University of Alberta

Electrical Stimulation Techniques to Restore Bladder and Sphincter Control after Spinal Cord Injury

by

Robert Gaunt ©

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– to Heather,

for your patience, and unwavering support

Abstract

In the aftermath of a spinal cord injury (SCI) bladder control is frequently impaired to the point where it becomes a primary medical concern. Since constant management of a dysfunctional bladder is required for the rest of the person's life, there is an urgent need to improve on current techniques such as clean intermittent catheterization. The work presented in this dissertation is focused on developing electrical stimulation techniques to restore bladder control that are suitable for a clinically relevant neuroprosthesis. The four studies presented herein address the following topics: i) the viability of intraspinal microstimulation (ISMS) as a technique to restore micturition after SCI; ii) relative recruitment orders of afferent and efferent axons in the spinal cord and the implications for the mechanisms of ISMS; iii) the efficacy of using transcutaneously coupled electrical stimulation to deliver high-frequency pulse trains to the pudendal nerve to block contractions of the external urethral sphincter; iv) the effect of anesthesia on functionally relevant parameters for high-frequency stimulation.

In these studies we demonstrated that ISMS is unlikely to form the basis of a neuroprosthesis for bladder control in the near future. Intraspinal microstimulation is a technically challenging approach, primarily frustrated at present by the lack of suitable electrode technologies for chronic implantation in the sacral spinal cord. The second study demonstrated that ISMS in the lumbar spinal cord directly recruits afferent axons at lower stimulus strengths than motoneurons. In addition, stimulation through single electrodes elicits afferent activity that spreads rostro-caudally in the cord, reflexly activating motoneurons in the entire lumbar enlargement. High-frequency stimulation of the pudendal nerve via a transcutaneous stimulus delivery system was shown to be an effective means of eliminating urethral sphincter contractions in both anesthetized and conscious animals. This could be used to promote micturition after SCI that is otherwise impeded by sphincter hyperreflexia. The final study demonstrated that high-frequency stimulation appears to have opposing effects on nerve conduction with and without anesthesia when the stimulation frequency is approximately 3 kHz. This is an unexpected complication for studies of high-frequency blockade of mammalian nerve which hitherto have been performed during anesthesia.

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List of Abbreviations

Abbreviation	Definition
AMS	American Medical Systems
ANOVA	analysis of variance
BFa	biceps femoris anterior
BFp	biceps femoris posterior
BION^{TM}	BIOnic Neurons
CNS	central nervous system
Cx	cervical segment number x
DC	direct current
DGC	dorsal gray commissure
DR	dorsal root
EMG	electromyography
ENG	electroneurography
EUS	external urethral sphincter
GABA	γ-aminobutyric acid
HF	high-frequency
HFS	high-frequency stimulation
im	intramuscular
ISMS	intraspinal microstimulation
IUS	internal urethral sphincter
iv	intravenous
LF	low-frequency
LFS	low-frequency stimulation
LG	lateral gastrocnemius
LUT	lower urinary tract
Lx	lumbar segment number x
MG	medial gastrocnemius
MN	motoneuron
NIH	National Institutes of Health
NMDA	N-methyl-D-aspartic acid
PAD	primary afferent depolarization
PMC	pontine micturition center
PN	pudendal nerve
SARTa	sartorius anterior
sc	subcutaneous
SCI	spinal cord injury
SD	standard deviation

Abbreviation	Definition
SMa	semimembranosus anterior
SPN	sacral parasympathetic nucleus
SRS	stimulus router system
Sx	sacral segment number x
TA	tibialis anterior
Tx	thoracic segment number x
VL	vastus lateralis

Chapter 1

Introduction

> *"After all, it is all of these autonomic functions that we take for granted when we have them and that dominate our lives when we lose them."*
>
> – Kim Anderson (2006)

The neural control of the bladder and sphincter is surprisingly complex. Conceptually, the sole function of the lower urinary tract is to temporarily store fluid and then release it at an appropriate time. While an engineer would have designed this system in a straightforward manner, nature has provided mammals with a system that is full of intricacies. It is neither fully reflexive, nor fully voluntary, but requires a perfect balance of both to operate normally. In fact, coordination between the somatic nervous system and both the sympathetic and parasympathetic branches of the autonomic nervous system are required to carry out the normal duties of this system. No less than seven identifiable reflexes, both spinal and supraspinal, in addition to conscious voluntary control are involved in simply filling and emptying a bag. Given its conceptually simple role, it is not surprising that this system can be overlooked in its importance to health and quality of life. However, anyone who has suffered the consequences of impaired bladder or sphincter function knows the tremendous impact that it can have on their life.

1.1 Spinal cord injury and bladder function

Every year, approximately 13,000 people in the United States and Canada suffer a spinal cord injury (SCI) while approximately 250,000 people live with SCI (Go et al., 1995). Although SCI presents many challenging medical problems, not the least of which is paralysis, disruption to the autonomic nervous system requires immediate, thorough and lifelong attention to manage. The importance of managing autonomic function for quality of life has been addressed by a survey of spinal cord injured people in which bladder function was ranked as being the second most desired area of improvement by people living with SCI (Anderson, 2004). Inadequate post-injury management of lower urinary tract function can lead to complications including renal failure, which for the early parts of the 20^{th} century was the leading cause of mortality after SCI. Fortunately this is no longer the case (Frankel et al., 1998). However, complications resulting from impaired lower urinary tract function, usually urinary tract infections, are the most common cause of rehospitalization after SCI and account for the second highest number of bed-days for readmitted patients (Middleton et al., 2004; Savic et al., 2000). Clearly, current techniques to manage bladder dysfunction have room for improvement.

1.2 Lower urinary tract anatomy and physiology

The lower urinary tract has two functions; storing urine (continence) and expelling urine from the body (micturition). The lower urinary tract consists of the bladder, bladder neck, urethra and sphincters and is innervated by the somatic nervous system and both the sympathetic and parasympathetic branches of the autonomic nervous system.

1.2.1 Anatomy

Sympathetic innervation of the bladder muscle (detrusor) arises from spinal segments T11-L2 in humans with fibers passing through the inferior mesenteric ganglia and hypogastric plexus before innervating the detrusor muscle via the hypogastric nerve. Parasympathetic innervation of the detrusor arises from the sacral parasympathetic nucleus (SPN) in spinal segments S1-S3 (Morgan et al., 1979; Vanderhorst and Holstege, 1997) with neurons traveling in the pelvic nerve to the pelvic ganglia on the bladder where they make synaptic contact with postganglionic neurons. The SPN in turn receives descending connections from the pontine micturition center. Afferent innervation of the bladder is primarily via the pelvic nerve (Morgan et al., 1981) although some afferent fibers are present in the hypogastric nerve.

The anatomy and terminology of the urethral sphincter complex is disputed, but the classical description includes two sphincters: the smooth muscle internal urethral sphincter (IUS) and the skeletal muscle external urethral sphincter (EUS). The IUS is integrated in the bladder neck, innervated by the hypogastric nerve and under the control of the sympathetic branch of the autonomic nervous system. The EUS, which lies distal to the IUS is under the control of the somatic nervous system. Both afferent and efferent innervation of the EUS as well as afferent innervation of the urethra itself is via the pudendal nerve arising from spinal segments S1-S3. The cell bodies of motoneurons innervating the EUS form Onuf's nucleus in the lateral aspect of the ventral horn. EUS motoneurons receive inhibitory input from GABAergic interneurons in the dorsal gray commissure (DGC) and excitatory inputs from supraspinal centers (Blok et al., 1997).

Voluntary switching between continence and micturition and the coordination between the bladder and sphincters are controlled in the brainstem (Barrington, 1921, 1925). While many supraspinal centers are implicated in the control of micturition (Zermann et al., 1998), the primary projection to the spinal cord arises from the pontine micturition center (PMC).

1.2.2 Physiology

In the continent state, the sympathetic nervous system actively inhibits detrusor contractions by the action of norepinepherine on β_2 adrenergic receptors in the bladder wall while causing tonic contraction of the IUS by the action of norepinepherine on α_1 receptors. As the bladder fills, stretch sensitive mechanoreceptors in the bladder wall transmit a sense of fullness to both spinal and supraspinal centers. Once the decision to urinate is reached, activity in the sympathetic nervous system decreases, the EUS is voluntarily relaxed and acetylcholine, released by the parasympathetic nervous system, acting on muscarinic receptors in the bladder wall causes a contraction of the detrusor. This coordinated activity, controlled by the PMC, results in micturition.

In addition to voluntary central control, spinal reflexes exist that promote both continence and micturition. It is these reflexes, when released from descending controls, and modified in their strength and association with each other, that cause the typical pathophysiology of lower urinary tract function associated with SCI. In the intact state, increases in bladder volume initiate both spinal and brainstem reflexes that promote continence by increasing activity in the EUS and relaxing the bladder (Barrington, 1931). After SCI, increases in bladder volume initiate reflexive bladder contractions accompanied by reflexive co-contractions of the EUS. This condition, detrusor-sphincter dyssynergia, can cause high intravesical pressures leading to ureteric reflux and upper urinary tract deterioration. A spinal reflex that causes coordinated contraction of the bladder and relaxation of the EUS both before and after SCI has been demonstrated by afferent stimulation of branches of the pudendal nerve arising from the urethra (Barrington, 1931, 1941; Boggs et al., 2005; Shefchyk and Buss, 1998). In addition, neural circuitry exists in the sacral spinal cord that has been shown to decrease EUS activity via the action of inhibitory interneurons in the DGC that project to EUS motoneurons (Blok et al., 1997, 1998; Buss and Shefchyk, 2003; Carter et al., 1995). It is thought that activation of these DGC interneurons via descending projections from the PMC is the mechanism by which the EUS is inhibited during normal micturition (Blok et al., 1997).

1.3 Dissertation summary and outline

The primary aim of the studies described in this dissertation was to develop electrical stimulation techniques that can be used to improve bladder and sphincter control in people with SCI. Four experimental studies are presented in this dissertation and are focused on two unique electrical stimulation techniques: stimulation of the sacral spinal cord with penetrating microwires and high-frequency stimulation of the pudendal nerve. Two different studies are described for each of these stimulation paradigms. The first study focuses on the use of the electrical stimulation paradigm to improve bladder and sphincter function while the second study addresses a specific neurophysiological question relating to the technique itself. Throughout the course of this work, a primary focus was to evaluate the stimulation techniques based on the likelihood of their translation to clinical treatments in the near future.

Perhaps as a result of the apparent simplicity of bladder control and the serious medical issues associated with impaired bladder function, many electrical stimulation techniques have been investigated in the past with the aim of improving bladder function. Chapter 2 presents a detailed summary of the numerous attempts to use devices to improve bladder and sphincter function after SCI. Devices, specifically catheters, play an important role in the daily regimen of bladder management for most people with SCI. The high incidence of complications associated with the use of catheters, and the fact that the spinal segments involved in lower urinary tract control remain intact in most cord-injured people, have been motivating factors to pursue research into devices that could harness the nervous system to provide greater control over lower urinary tract function. The sites that have been tested include: inside the bladder, bladder wall, thigh, pelvic floor, dorsal penile nerve, pelvic nerve, tibial nerve, sacral roots, sacral nerves and spinal cord. Catheters and sacral root stimulators are two techniques whose efficacy is well established. Modifications to sacral root stimulation including posterior root stimulation, anodal blockade and high-frequency blockade as well as new techniques including intraspinal microstimulation, urethral afferent stimulation and injectable microstimulators are also discussed. This chapter primarily reviews the use of electrical stimulation techniques to improve bladder and sphincter function after SCI although many of these techniques have also been investigated to manage bladder and sphincter dysfunction secondary to other medical conditions.

The first electrical stimulation technique investigated for this dissertation, presented in Chapter 3, involved implanting arrays of fine wires into the sacral spinal cord to target intraspinal structures associated with bladder control. This method of stimulating intraspinal structures was an extension of an earlier set of studies on intraspinal stimulation to restore micturition after SCI (Carter et al., 1995; Grill et al., 1999; Nashold et al., 1971) and the physiological substrates required for an intraspinal neuroprostheses had been demonstrated in acute experiments. We wanted to test the viability of using intraspinal microstimulation (ISMS) to elicit micturition in chronically implanted conscious animals with intact or transected spinal cords. Despite observing both bladder contractions and EUS inhibition, incomplete micturition only occurred in one animal. Ultimately, ISMS using arrays of discrete microwires failed to elicit micturition and we felt that other electrical stimulation techniques hold more promise for developing a clinically viable neuroprosthesis for bladder control.

During the course of ISMS experiments for bladder control, as well as previous work in ISMS for locomotion, the question of which intraspinal neural structures are actually activated by ISMS arose. Chapter 4 describes experiments in which the relative recruitment order of motoneurons and sensory afferents was measured in response to ISMS. Intraspinal microstimulation was applied through microwires implanted in the dorsal horn, intermediate region and ventral horn of the L7 segment of the spinal cord in four acutely decerebrated cats, two of which had been chronically spinalized. Activation of sensory axons was detected with electroneurogram (ENG) recordings from dorsal rootlets. Activation of motoneurons was detected with electromyogram (EMG) recordings from hindlimb muscles. Sensory axons were nearly always activated at lower stimulus levels than motoneurons irrespective of the stimulating electrode location. Electromyogram response latencies decreased as ISMS stimulus intensities increased, suggesting that motoneurons were first activated transsynaptically and then directly as intensity increased. Intraspinal microstimulation elicited antidromic activity in dorsal root filaments with entry zones over nearly the entire lumbar enlargement. This result shows that action potentials elicited in localized terminal branches of afferents spread antidromically to all terminal branches of the afferents and transsynaptically excite motoneurons and interneurons far removed from the stimulation site. This may help explain how focal ISMS can activate many motoneurons of a muscle, even though they are distributed in long thin columns.

As a result of the demonstration that ISMS was unlikely to form the basis of a clinical neuroprosthesis for bladder control in the near future, attention was turned to stimulation of the pudendal nerve using a new neuroprosthetic stimulus delivery system. Chapter 5 demonstrates that using a transcutaneously coupled stimulus delivery system, high-frequency pulse trains could be used to block contractions of the EUS in acute experiments as well as in a chronically implanted animal. High-frequency stimulation (kHz range) has been shown to elicit a fast-acting and reversible block of action potential propagation in peripheral nerves and may be useful in treating detrusor-sphincter dyssynergia which often prevents the normal expulsion of urine in people with SCI.

Chapter 6 continues on the theme of high-frequency blocking and demonstrates that anesthetics can have an effect on the appearance of high-frequency blocking in various experimental preparations. In spite of a number of computer modeling studies (Bhadra et al., 2007; Kilgore and Bhadra, 2004; Tai et al., 2005a,b; Williamson and Andrews, 2005; Zhang et al., 2006), the mechanism of high-frequency blocking is not completely understood, and the ranges of stimulation parameters that reliably elicit a nerve block are not completely understood. In this study we examined the responses to high-frequency stimulation in two different experimental preparations with and without anesthesia. These data show that high-frequency stimulation can elicit a complete nerve conduction block in unanesthetized and conscious animals at 16 kHz, but not at 3 kHz. Chapter 7 contains a summary of the experimental findings presented in this dissertation, a general discussion of the implications of these results and suggestions for future directions in the efforts to develop electrical stimulation techniques for bladder control.

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Chapter 2

Control of urinary bladder function with devices: successes and failures*

> *"Now my own suspicion is that the Universe is not only queerer than we suppose, but queerer than we can suppose."*
>
> – J.B.S. Haldane

2.1 Introduction

People with spinal cord injury face many challenging medical problems. Inadequate post-injury management of lower urinary tract dysfunction can lead to many complications including renal failure. This used to be the leading cause of death after spinal cord injury, but has dropped to fourth position in recent decades (Frankel et al., 1998) with improved treatment methods (Jamil, 2001). However, complications of the genitourinary system, primarily urinary tract infections, are the most common cause of rehospitalization after spinal cord injury and account for the second highest number of bed-days for readmitted patients (Middleton et al., 2004; Savic et al., 2000). While management of lower urinary tract dysfunction with devices, primarily catheters, has reduced mortality after spinal cord

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injury, the high incidence of complications is largely due to the limited success that these devices or other treatment modalities have had in restoring normal function to the neurogenic bladder. In addition to these clinical considerations, effective management of lower urinary tract dysfunction is generally outranked in its importance to patients only by the desire for hand function in people with quadriplegia and sex function in people with paraplegia (Anderson, 2004). These factors provide an impetus to develop improved methods of managing lower urinary tract dysfunction after spinal cord injury.

2.1.1 Lower urinary tract control

The lower urinary tract has two functions: storing urine (continence) and voiding urine (micturition). The lower urinary tract is innervated by the somatic nervous system and both the sympathetic and parasympathetic branches of the autonomic nervous system. Efferent parasympathetic innervation of the detrusor, the muscular layer of the bladder, arises from preganglionic neurons in the sacral (S) parasympathetic nucleus in spinal segments S2-S4. The preganglionic neurons send axons via the pelvic nerve to the pelvic plexus where they synapse with ganglionic neurons. Afferent innervation of the bladder is also primarily via the pelvic nerve. Efferent somatic innervation of the external urethral sphincter arises from motoneurons in Onuf's nucleus in spinal segments S1-S3. These efferent axons as well as the afferents of the external urethral sphincter and urethra travel via the pudendal nerve. As the bladder fills during the storage phase, stretch sensitive mechanoreceptors in the bladder wall transmit a sense of fullness to both spinal and supraspinal centers. Once the decision to void is reached, the external urethral sphincter is voluntarily relaxed and parasympathetic activity causes detrusor contractions. This synergistic activity, coordinated by the pontine micturition center, results in micturition (Barrington, 1921, 1925). More details on the anatomy and physiology of the lower urinary tract can be found in de Groat (1993) and de Groat et al. (2001).

After spinal cord injury, supraspinal coordination from the pontine micturition center is lost leading to lower urinary tract dysfunction. Sacral spinal cord or cauda equina lesions generally lead to an areflexive bladder and sphincter paralysis. Suprasacral lesions however, spare sacral spinal reflexes, and after a period of shock, reflexive bladder contractions often occur at low bladder volumes. This condition, called detrusor hyperreflexia or neurogenic detrusor overactivity (reviewed in Yoshimura, 1999), is often accompanied by reflexive co-contractions of the external urethral sphincter. This combination, termed detrusor-sphincter dyssynergia (Andersen and Bradley, 1976; Blaivas et al., 1981) leads to incontinence, inefficient voiding with high residual volumes and high intravesical pressures which in turn leads to ureteric reflux and upper urinary tract deterioration.

2.1.2 Why devices?

Any treatment for lower urinary tract dysfunction after spinal cord injury should create a bladder capable of storing large volumes of urine at low pressure, prevent incontinent episodes, and allow periodic evacuation of urine at low pressure. Surgical treatments, such as bladder augmentation using a section of intestine, ameliorate the problem of hyperreflexia and low storage volume (Hollander and Diokno, 1993), while sphincterotomies (cutting into the external urethral sphincter) improve detrusor-sphincter dyssynergia (Reynard et al., 2003). Anticholinergic medications are frequently used to relax the hyperreflexive bladder but have undesirable side effects including a dry mouth and blurred vision (Wein, 1998). These treatments address the symptoms of the neurogenic bladder so that storage and evacuation of urine is achieved without upper urinary tract damage, but they do not address the fundamental loss of control associated with spinal cord injury.

Devices present attractive alternatives to the management of lower urinary tract dysfunction after spinal cord injury as they attempt, at least partly, to restore the control of the neurogenic bladder. Additionally, while devices are locally invasive to varying degrees, they do not generally cause systemic complications as do pharmacological treatments. Surgical procedures such as the ones described above are usually irreversible and subsequently limit patients to a specified course of treatment while possibly excluding new techniques. The devices described in this review, and those under development do not generally cause irreversible changes and therefore do not prevent patients from taking advantage of improved treatments in the future.

Many review articles have been published that focus on devices for bladder control (Grill et al., 2001; Groen and Bosch, 2001; Jamil, 2001; Jezernik et al., 2002; Lee, 1997; Middleton and Keast, 2004; Rijkhoff, 2004b; Rijkhoff et al., 1997b; Schmidt, 1983; Talalla et al., 1987; van Balken et al., 2004; Van Kerrebroeck, 2002), so no attempt will be made to provide detailed descriptions of each of these methods here. Rather, we will summarize the methods that have been devised over the years for device-based management of the neurogenic bladder secondary to spinal cord injury and summarize current research on those devices and methods that are likely to affect the field in the future. Additionally, we will attempt to identify the reasons that many methods and devices have ultimately been unsuccessful, sometimes in spite of good clinical results. Finally, we will summarize the problems that we feel should be addressed to improve the effectiveness and adoption of devices in the management of the neurogenic bladder. Both mechanical and electrical devices will be described as they have met with different levels of success and failure and have the potential to offer solutions to a variety of the problems faced by people with spinal cord injury.

2.2 Mechanical devices for control of the lower urinary tract

2.2.1 Catheters

The use of catheters to manage urinary retention dates back to ancient Egypt (reviewed in Nacey and Delahunt, 1993). During World War I, up to 80% of patients with spinal cord injury died shortly after injury due to complications arising from the neurogenic bladder (Kennedy, 1946). However, improved management of the lower urinary tract using catheters during World War II (Kennedy, 1946) and especially Guttmann's technique of sterile intermittent catheterization (Guttmann and Frankel, 1966) helped reduce this figure significantly. Sterile intermittent catheterization was eventually modified to non-sterile clean intermittent catheterization for reasons of practicality (Comarr, 1972; Lapides et al., 1972), and this technique, along with generally improved medical care, has caused urinary tract dysfunction to fall from the primary cause of death (22%) for patients injured between 1943-1972 to the fourth most common cause of death (9%) for patients injured between 1973-1990 (Frankel et al., 1998).

Chronically indwelling urethral and suprapubic catheters, condom catheters and clean intermittent catheterization are common forms of catheterization currently used in the management of spinal cord injury patients. Each method has its own advantages and disadvantages (reviewed in Selzman and Hampel, 1993), but clean intermittent catheterization is the form of bladder management least likely to lead to complications (Weld and Dmochowski, 2000). Clean intermittent catheterization is generally the most prescribed form of bladder management at hospital discharge (Cardenas et al., 1995), and although a number of reports suggest that there is a trend for some people to switch to other methods (Cardenas et al., 1995; Weld and Dmochowski, 2000), a more recent study suggests that this trend may be reversing (Hansen et al., 2004). However, only 30% of cord-injured people using clean intermittent catheterization remain free of urinary tract infections. Clean intermittent catheterization requires good hand function, preventing people with tetraplegia and impaired hand function as well as some people with paraplegia from performing this procedure themselves (Dahlberg et al., 2004; Selzman and Hampel, 1993).

The critical role of catheter technology and techniques in the management of lower urinary tract dysfunction after spinal cord injury cannot be overstated. The simplicity and clinical efficacy of catheters in increasing life expectancy in people with cord injury make them arguably the single most important device for these people. However, the high incidence of urinary tract infections and other complications associated with catheter use presents a continuous burden on patients and the medical system. This, and the desire of people with spinal cord injury for improved methods (Anderson, 2004), is a motivation for new device development.

2.2.2 Artificial sphincters

The concept of an artificial urethral sphincter was first proposed by Foley (1947) to treat urinary incontinence. The artificial urethral sphincter developed by Scott, Bradley and Timm (Scott et al., 1974; Timm et al., 1974) has developed into the commercially available AMS 800 artificial sphincter (American Medical Systems, Minnetonka, MN, USA) (reviewed in Hajivassiliou, 1998). The AMS 800 uses a pump to deflate a cuff placed around the bladder neck or urethra by transferring fluid to a pressure-regulated reservoir. The cuff re-inflates automatically over a period of several minutes. Of reported studies using the AMS 800 including 2606 subjects, 73% achieved full continence, 14% experienced device failure, 4.5% experienced infections and 11.7% experienced urethral erosion from excessive pressure placed on the urethra by the cuff (Hajivassiliou, 1998). Artificial urethral sphincters are primarily used to treat patients with post-prostatectomy incontinence, but have been successful in managing incontinence with other etiologies as well (Petrou et al., 2000). It was originally suggested that detrusor hyperreflexia was a contraindication for artificial urethral sphincter implantation as high intravesicular pressures may cause deflation of the pressure-regulated cuff (Scott et al., 1974). However, artificial urethral sphincters have been implanted in spinal cord injury patients with an overall success rate of 70% (Light and Scott, 1983), though device removal due to infections was high (24%). Currently, artificial urethral sphincters are not commonly used to manage incontinence after spinal cord injury, but can be useful in people with lesions leading to a flaccid bladder and sphincter.

2.2.3 Urethral stents

Urethral stents were first developed to treat urethral strictures, but shortly after, their use in spinal cord injury patients with detrusor-sphincter dyssynergia leading to hydronephrosis and vesicoureteric reflux was described (Shaw et al., 1990). Urethral stents were proposed as an alternative to sphincterotomies, the primary surgical treatment for patients with detrusor-sphincter dyssynergia. Sphincterotomies are generally irreversible and can cause hemorrhage, erectile dysfunction, bladder neck stenosis or stricture (reviewed in Reynard et al., 2003). Urethral stents are inserted into the urethra and mechanically hold the external urethral sphincter open. After sphincterotomy, or implantation of a urethral stent, most cord-injured people must wear a collection device such as a condom catheter as the continence mechanism of the urethra is defeated.

Several different urethral stent designs have been tested in various trials including the UroLume® (American Medical Systems, Minnetonka, MN, USA) (Chancellor et al., 1999b), Memokath® (Doctors & Engineers A/S Ltd., Kvistgaard, Denmark) (Hamid et al., 2003; Low and McRae, 1998), Memotherm® (Bard Corp., Covington, GA, USA) (Juan Garcia et al., 1999) and Ultraflex® (Boston

Scientific Corp., Natick, MA, USA) (Chartier-Kastler et al., 2000). The UroLume, Memotherm and Ultraflex are flexible wire mesh tubes while the Memokath is a helically wound wire. The devices are inserted into the urethra and positioned in the region of the external urethral sphincter where the wire becomes largely covered by urothelium over time. The UroLume is the most well studied of these devices and has similar results in terms of urodynamic parameters and incidence of urinary tract infection to sphincterotomies, but requires less hospitalization and is potentially reversible (Chancellor et al., 1999a). A 5-year multi-center trial of the UroLume in 160 cord-injured subjects showed that the treatment was successful in 84%, while 15% required explantation. Complications such as device migration were most common in the first 3 months (Chancellor et al., 1999b). Although explantation of the stent was possible, it has presented a variety of challenges (Chancellor et al., 1999b; Wilson et al., 2002). The Memokath was found to be suitable for short-term implantation only as most devices fail within two years (Hamid et al., 2003) and complications including migration, autonomic dysreflexia and stone formation on the stent can occur (Low and McRae, 1998). However, explantation of this device is much simpler than the UroLume due to its helical design and thermosensitive material which, when cooled with saline, becomes soft and uncoils, making this device useful for acute management of detrusor-sphincter dyssynergia (Hamid et al., 2003).

Urethral stents represent a clinically successful device for management of detrusor-sphincter dyssynergia in people with spinal cord injury. Although stents do not restore normal control of the sphincter, their efficacy, simplicity and potential reversibility makes them an attractive option for people who would otherwise receive an irreversible sphincterotomy (Chancellor et al., 1999b).

2.2.4 Intraurethral pump

In 1997, Nativ et al. (1997) described a device incorporating a miniature valve and pump that could be inserted into the urethra to control both continence and voiding in women. The In-Flow^{TM} intraurethral pump (SRS Medical Systems, Inc., Billerica, MA, USA) is designed to manage chronic urinary retention caused by an atonic bladder or urethral dysfunction. The device secures itself in the urethra by means of flexible fins that open in the bladder and a flange at the external urethral

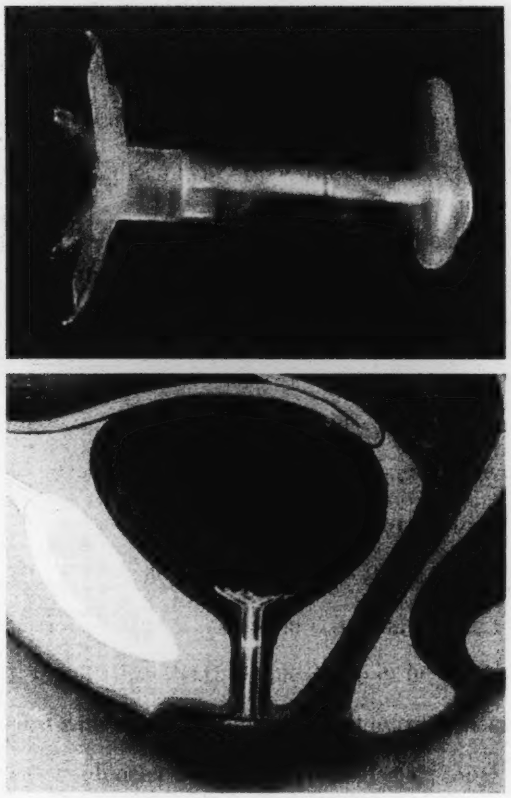

Figure 2.1: *The In-Flow*^{TM} *intraurethral pump*

(A) Photograph showing the unfolded petals that secure the device in the urethra and prevent migration. (B) Diagram showing the placement of the device in the urethra. Adapted from Madjar et al. (1999) and Schurch et al. (1999).

meatus (see Figure 2.1). The device is controlled by a remote activator that is placed over the pubic area and is magnetically coupled to the pump. Once activated, the turbine actively pumps urine out of the bladder at a rate of 6-12 mL/s until the bladder is empty. The device is easily inserted by a physician and can be removed by the patients if they wish. The device is designed to be replaced every month, but successful usage to an average of 90 days has been reported, at which time the device can become fouled by salt deposits (Madjar et al., 1999).

In a study of 18 women with spinal cord injury and hyporeflexive bladders, only 6 continued to use the device at follow-up (mean 9.6 months) (Schurch et al., 1999). Discomfort, incontinence, urinary tract infections, technical failures, urethral dilation and the possibility of long-term urethral damage were cited as reasons why this device was unsuitable for chronic use. Studies in 60 (Mazouni et al., 2004) and 92 (Madjar et al., 1999) patients with voiding dysfunction from various etiologies reported success rates of 50% with average follow-up times of 3 and 7.6 months respectively. Most of those patients that adopted this device for long-term usage were previously dependent on clean intermittent catheterization and preferred the convenience of this device. Intraurethral pumps are very interesting from a technical viewpoint and further investigation with clearer indications for use, such as complete spinal cord injury, atonic bladder and previous dependence on clean intermittent catheterization may improve the success rate among people with spinal cord injury.

2.3 Electrical stimulation devices for control of the lower urinary tract

While mechanical devices are necessarily limited to treating symptoms of the neurogenic bladder, electrical stimulation techniques allow devices to be created that can exert control over spared muscles and their neural control systems. Electrical current, passed between two electrodes, can be used to generate action potentials in surviving neurons in the spinal cord or peripheral nerve below the lesion in spinal cord injury patients. These artificially generated action potentials can lead directly to muscular contraction or they can modulate the activity of neuronal networks and reflex pathways (termed neuromodulation).

The discussion below of devices and techniques that have been developed to control the lower urinary tract is organized by the location of stimulation electrodes rather than by the neurophysiological mechanisms on which the devices operate or by their intended function. Five primary locations can be identified where electrical stimulation electrodes can be placed: on or in the bladder, on the skin, peripheral nerve, sacral roots, and in the spinal cord itself. Figure 2.2 shows the various stimulation locations for devices discussed throughout this review.

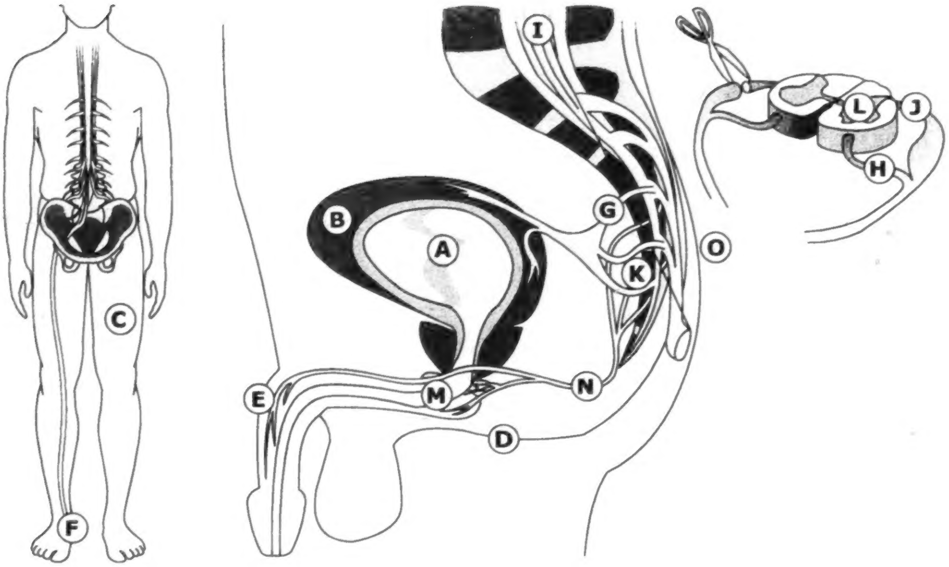

Figure 2.2: *Electrode locations for controlling the lower urinary tract*

The locations are numbered primarily by the order in which they are discussed in the text. The location for a posterior rhizotomy is also indicated. (A) Intravesical, (B) Bladder wall, (C) Thigh, (D) Pelvic floor, (E) Dorsal penile nerve, (F) Tibial nerve, (G) Pelvic nerve, (H) Intradural sacral anterior root, (I) Extradural mixed sacral root, (J) Intradural sacral posterior root, (K) Sacral nerve, (L) Spinal cord, (M) Intraurethral, (N) Pudendal nerve, (O) Sacrum.

2.3.1 Electrical stimulation of the bladder

Intravesical stimulation

Intravesical electrical stimulation was the first attempt at treating bladder dysfunction using electrical stimulation. In 1878, M.H. Saxtorph described a technique in which stimulation between a catheter-mounted electrode, passed into the bladder to act as the cathode (see Figure 2.2A), and a suprapubically placed indifferent electrode, were used to treat urinary retention caused by an underactive bladder (reviewed in Madersbacher, 1990). Intravesical electrical stimulation is essentially a neuromodulation therapy intended to reinforce the weak functioning of existing neural micturition pathways by stimulating mechanoreceptors in the bladder wall to facilitate reflex bladder contractions and improved sensation. Electrical stimulation is below the threshold required to elicit bladder contractions directly via stimulation of the efferent portion of the pelvic nerve or of the detrusor myocytes themselves. Acute studies in rats and cats have confirmed the hypothesis that intravesical electrical stimulation acts by stimulating stretch-sensitive mechanoreceptors in the wall of the bladder that reflexively cause contractions of the bladder (Ebner et al., 1992).

Few reports of intravesical electrical stimulation studies in people with spinal cord injury exist, but one dealing specifically with subjects with incomplete spinal cord injury reported improvements in bladder sensation, detrusor contraction and residual volumes in almost all subjects (Madersbacher et al., 1982). A retrospective study on the effectiveness of intravesical electrical stimulation for people with spinal cord injury by the same author indicated that one third of the subjects experienced improvements in sensation, detrusor contractility and voluntary control. This occurred only in individuals with preserved pain sensation in the S2-S4 dermatomes (Madersbacher, 1990). This would seem to be the only predictor of the efficacy of this therapy. Additionally, patients require many hours of treatment before the effectiveness of intravesical electrical stimulation can begin to be evaluated and the positive results reported by some investigators (Kaplan, 2000) have not been repeatable by others (Decter, 2000). While intravesical electrical stimulation has been used to treat patients with spinal cord injury, recent studies have focused on children with underactive bladders (Gladh et al., 2003). Intravesical electrical stimulation appears ultimately unattractive as a clinical technique to improve micturition in people with spinal cord injury as it only appears to work in some with incomplete spinal cord injury, requires long treatments before effectiveness can be evaluated and the results have not been repeatable amongst investigators.

Bladder wall stimulation

Electrical stimulation of the exterior surface of the bladder (see Figure 2.2B) was first studied in the early 1950's (Boyce et al., 1964). This marked the beginning of the development of electrical stimulation devices to elicit voiding directly, in response to the high morbidity and mortality associated with catheterization (Bradley et al., 1962). Several groups developed implanted stimulators inductively coupled to external transmitters with variations in the design, placement and number of electrodes (Bradley et al., 1962; Hald et al., 1967; Magasi and Simon, 1986; Merrill and Conway, 1974; Stenberg et al., 1967; Susset and Boctor, 1967). Initial animal experiments demonstrated that dogs with spinal cord transections were able to void regularly using the implanted stimulators without requiring additional procedures (Bradley et al., 1963, 1962; Kantrowitz and Schamaun, 1963). However, results in spinal cord injury patients implanted with these stimulators were much less successful (Bradley et al., 1963; Hald et al., 1967; Merrill and Conway, 1974; Stenberg et al., 1967; Susset and Boctor, 1967). The primary reason that these people were unable to void was that stimulation currents high enough to generate useful bladder contractions spread to surrounding structures causing co-activation of the external urethral sphincter and pelvic floor musculature. It was noted that the canine bladder is primarily an abdominal organ, whereas the human bladder is a pelvic organ and is in close proximity to the pelvic floor musculature, increasing its susceptibility to contraction by current spread (Bradley et al., 1963).

Because of this problem, experimental and clinical work was then directed toward obtaining sufficient contraction of the bladder while limiting current spread. Tape electrodes and more powerful stimulators successfully elicited micturition, but infection and technical failures prevented evaluation of their long-term effect (Bradley et al., 1963). Experience with the Avco stimulator, in which individual wires were embedded into the bladder wall, were also hampered by activation of urethral and pelvic floor musculature (Hald et al., 1967; Stenberg et al., 1967). Another stimulator design, the Mentor bladder stimulator, used two helical wire electrodes sewn into the bladder wall. This was successful in two of five people with upper motoneuron lesions, but required subarachnoid injections of phenol to abolish electrically-induced detrusor-sphincter dyssynergia that otherwise prevented micturition (Merrill and Conway, 1974). Susset and Boctor (1967) reported a successful implant that incorporated 8 disc electrodes around the dome of the bladder in a person with a complete lower motoneuron lesion. These investigators considered upper motoneuron lesions to be a contraindication for implantation of these systems due to the unwanted activation of sphincter and pelvic floor muscles. The most successful report of bladder wall stimulation was made by Magasi and Simon (1986) in which 29 of 32 subjects with neurogenic bladder paralysis attained complete voiding with 8 disc electrodes implanted around the bladder (see Figure 2.3). However, the concomitant sphincter activation reported by most investigators, lead and electrode breakage, receiver malfunction, bladder perfora-

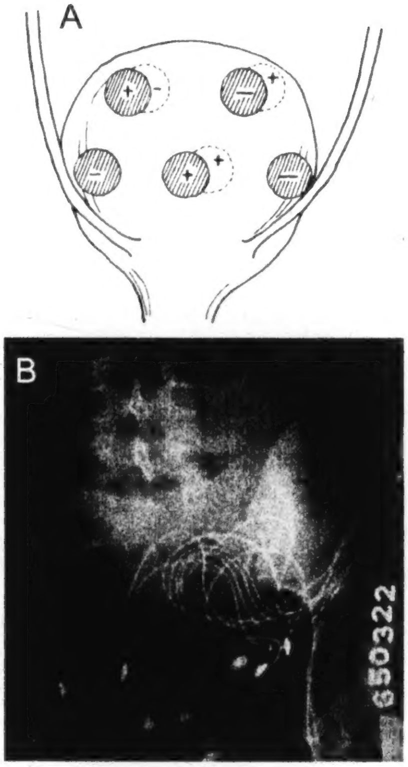

Figure 2.3: *Diagram and x-ray showing electrodes on the bladder wall*

This configuration was used by Susset and Boctor (1967). (A) The intended positioning of electrodes on the bladder. (B) Actual positions of electrodes around the bladder in one female subject. Reprinted from Susset and Boctor (1967) with permission from S. Karger AG, Basel.

tion and pain caused failure in most human studies. With the success of sacral root stimulation (see below) for restoring micturition in people with upper motoneuron lesions, and the multiple difficulties in achieving successful clinical results with bladder wall stimulation, recent work in this area has focused on people with lower motoneuron lesions who cannot benefit from sacral root stimulation (Walter et al., 1999).

2.3.2 Transcutaneous electrical stimulation

Thigh stimulation

In 1986 it was reported that electrical stimulation through surface electrodes over the thigh muscles (see Figure 2.2C) could cause changes in the urodynamic parameters of spinal cord injury patients (Wheeler et al., 1986). Stimulation was applied through bilateral quadriceps surface electrodes on a daily basis for 4-8 weeks. Some people exhibited persistent increases in bladder capacity and/or reductions in bladder pressure, while others experienced the opposite result. Another study, examining hamstring and quadriceps stimulation to reduce spasticity in cord-injured people, noted that 16 of 32 subjects became continent (Shindo and Jones, 1987) perhaps indicating a suppression of detrusor hyperreflexia. A more recent study examining urodynamic changes in response to thigh muscle stimulation showed that 8 of 14 subjects, including one person with spinal cord injury and neurogenic detrusor overactivity, increased their bladder volumes by >50% (Okada et al., 1998). However, no methods of identifying those people likely to respond positively to treatment exist. None of these studies noted any adverse side effects from the treatment.

The effects of electrical stimulation of the thigh muscles on the bladder may be mediated by limb afferents known to inhibit bladder contractions to prevent leakage during physical activity (Fall and Lindstrom, 1991), although other mechanisms have been proposed (Okada et al., 1998). Carry-over, observed with thigh stimulation, has also been observed with other electrical stimulation techniques (Fall and Lindstrom, 1991), and may be at least partially explained by mechanisms such as those proposed by Vodovnik (1981). Despite the simplicity of this approach, efficacy in some people and lack of adverse side effects, few studies of stimulation of the thigh muscles have been reported, and this technique does not appear to be widely used in practice. This is likely because many patients show no improvement, and those that may cannot be identified prior to treatment. Additionally, treatment requires a significant time investment and most people can achieve effective suppression of hyperreflexive bladder contractions with anticholinergic medications.

Pelvic floor maximal functional electrical stimulation

On the basis of a previous observation, Moore and Schofield (1967) decided to test the effectiveness of electrically induced maximal contraction of the pelvic floor musculature (see Figure 2.2D) to treat female patients with stress incontinence. Some people reported being cured after a single session and more reported a reduction in symptoms. Maximal functional electrical stimulation may involve the use of surface, vaginal, anal, penile, percutaneous or a combination of such electrodes to stimulate the pelvic floor musculature and pudendal nerve at the maximum tolerable threshold for subjects. This treatment can lead to long-lasting bladder inhibition in subjects with non-neurogenic bladder overactivity (Fall and Lindstrom, 1991). While maximal functional electrical stimulation is used in some clinical settings for treating incontinence in many patient groups (Geirsson and Fall, 1997), results in people with spinal cord injury for suppressing hyperreflexive bladder contractions are mixed (reviewed in Previnaire et al., 1998). Given the side effects of maximal functional electrical stimulation , including physical discomfort in people with incomplete spinal cord injury, possibly limiting the stimulation current to non-therapeutic levels (Previnaire et al., 1998), as well as psychological discomfort (van Balken et al., 2004), anticholinergic medications are often a more practical method to manage neurogenic detrusor overactivity.

Dorsal penile nerve stimulation

Stimulation of the dorsal penile nerve or clitoral nerve can inhibit detrusor activity. These nerves form the most superficial branch of the pudendal nerve and are therefore easily accessible. Detrusor inhibition by this means was first demonstrated scientifically using mechanical stimulation (penile squeeze) to suppress ongoing bladder contractions (Kondo et al., 1982). This effect has also been demonstrated using electrical stimulation with bipolar surface electrodes placed on the penis (Nakamura and Sakurai, 1984) (see Figure 2.2E). Given the success of this simple technique in inhibiting bladder contractions, its potential in treating subjects with detrusor hyperreflexia secondary to spinal cord injury has been examined by several groups. In one study of 6 spinal cord injury subjects with complete and incomplete cervical and thoracic lesions, inhibition of detrusor contractions during bladder filling was demonstrated in all subjects (Wheeler et al., 1992). Continuous stimulation at 5 pulses per second was sufficient to increase the volume at which reflexive bladder contractions first occurred during a cystometrogram by an average of 76% (range 26% to 150%) without side effects. Continuous stimulation of the genital nerves, however, may pose practical challenges for the design of a neuroprosthesis and would preclude measurement of bladder activity using peripheral nerve recording techniques (Jezernik et al., 2000). Kirkham et al. (2001) and Dalmose et al. (2003) therefore examined whether conditional stimulation of the dorsal penile nerve was sufficient to effect clinically useful inhibition of the detrusor. Stimulation lasting one minute was initiated by a rise in bladder pressure of 10 cmH2o during a cystometrogram and successfully inhibited bladder contractions while increasing bladder capacity by 144% (±127%) in all six spinal cord injury subjects studied (Kirkham et al., 2001). The effects of conditional stimulation on detrusor inhibition are robust amongst both male (Dalmose et al., 2003; Kirkham et al., 2001) and female (Dalmose et al., 2003) patients with a wide range of injury levels. In addition to inhibiting hyperreflexive bladder contractions, dorsal penile nerve stimulation can reduce blood pressure in people with high level spinal cord injuries (Lee et al., 2003). This may reduce the risks associated with autonomic dysreflexia often triggered by a full bladder or bowel.

Dorsal penile nerve stimulation is an active area of neuroprosthesis development due to the relative simplicity of the technique and its reliability and efficacy in people with spinal cord injury. Two recent reports describe attempts at moving dorsal penile nerve stimulation from the laboratory to clinical use. Lee and Creasey (2002) describe the application of a surface stimulation system to a person with an incomplete cervical (C)6 injury who experienced episodes of incontinence after sensing his full bladder, but before he was able to catheterize himself. Figure 2.4 shows the effect that conditional dorsal penile nerve stimulation had on bladder contractions in this subject. During home use for 3 weeks, this man applied stimulation when he sensed his bladder was full, allowing him time to perform successful catheterization. He continued to use the system after the trial was over because of its success and his confidence in it. Additionally, Fjorback et al. (2003) developed a portable device that measured bladder pressure and automatically stimulated the dorsal penile nerve to inhibit bladder contractions. This device used a catheter to measure bladder pressure, and as such

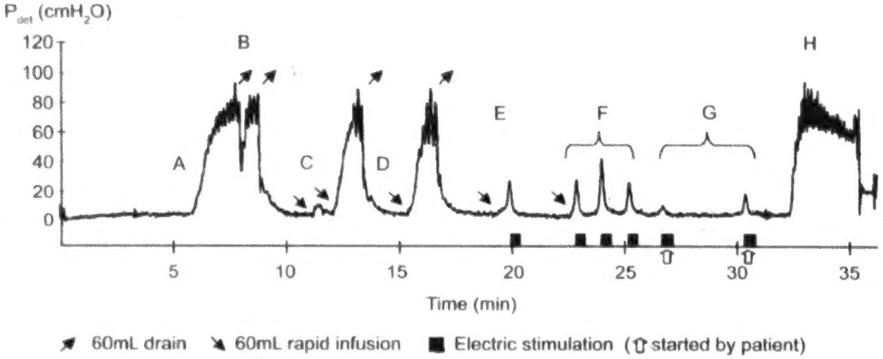

Figure 2.4: *Bladder pressure recording with and without dorsal penile nerve stimulation*

Reproducible reflexive bladder contractions were caused by rapid infusions of 60 mL of saline (C and D) and were abolished by withdrawal of the saline. Further provocations (E and F) resulted in small reflexive bladder contractions that were immediately abolished by stimulation of the dorsal penile nerve. During F and G, bladder pressure increases are hyperreflexive contractions caused by the high volume. The two stimulation periods shown by open arrows at the onset of termination of G indicate patient-initiated stimulation in response to sensation of bladder fullness. These suppressions of reflexive bladder contractions were better than the previous ones. Without stimulation, bladder contractions are not suppressed (H). Reprinted from Lee and Creasey (2002) with permission from the American Congress of Rehabilitation Medicine and the American Academy of Physical Medicine and Rehabilitation.

was impractical clinically, but did serve to demonstrate the feasibility of a closed-loop system to treat neurogenic detrusor overactivity.

2.3.3 Stimulation of peripheral nerve

Tibial nerve stimulation

In 1983, a report of investigations on nonhuman primates demonstrated that the amount of current required to cause detrusor inhibition with bipolar percutaneous anal sphincter stimulation could be reduced by changing the cathode to a surface electrode positioned over the posterior tibial nerve (McGuire et al., 1983). In the same study, similar results were observed with percutaneous tibial nerve stimulation alone (see Figure 2.2F). This target was chosen as it is the acupuncture point used to inhibit bladder contractions in Chinese medicine. This technique was successful in improving continence in 19 of 22 subjects, including four with spinal cord injury, although bladder contractions returned immediately once stimulation ceased.

More recent work with tibial nerve stimulation includes evaluation of the commercially available Urgent PC device (CystoMedix, Andover, MN, USA), formerly the Urosurge SANS device given FDA approval in 2000 (Govier et al., 2001; van Balken et al., 2001; Vandoninck et al., 2003). Encouraging results were reported in patients with overactive bladders although no spinal cord injury subjects were included in these studies. Two small-scale studies evaluating tibial nerve stimulation in subjects with spinal cord injury also reported reductions in incontinence caused by neurogenic detrusor overactivity, although the results in the subset of people with spinal cord injury are not stated in one study (Amarenco et al., 2003) and the other is a report from a single patient (Andrews and Reynard, 2003). Given the technical simplicity of this technique and its potential to suppress neurogenic detrusor overactivity, further experiments to determine efficacy in larger groups of spinal cord injury subjects would help determine if this technique should be pursued.

Pelvic nerve stimulation

Stimulation of the nerve supply to the bladder presents some potential advantages over bladder wall stimulation for people with spinal cord injury. Stimulation of the pelvic nerve (see Figure 2.2G), which contains the preganglionic parasympathetic fibers innervating the detrusor, should cause contraction of the entire detrusor at a much lower current than bladder wall stimulation (Hald, 1969). Pelvic nerve stimulation was shown to elicit bladder contractions in dogs, but co-activation of the sphincters prevented good micturition, especially in male dogs (Holmquist and Olin, 1968a,b). Sphincter activation was likely a reflex caused by stimulation of bladder afferents in the pelvic nerve. In addition, chronic stimulation was found to cause fibrosis of the pelvic nerve leading to reductions in bladder response over time (Hald, 1969). Application in humans was also frustrated because of the structure of the pelvic nerve. Whereas a distinct pelvic nerve exists in the cat and dog, parasympathetic innervation of the human bladder is distributed from the pelvic plexus arising from the pelvic nerves shortly after their exit from the sacral foramina (Wozniak and Skowronska, 1967), making placement of electrodes very difficult (Hald, 1969; Susset and Boctor, 1967). Despite this, brief reports describing mixed results with pelvic nerve stimulation in humans appeared in the 1970's

(Burghele, 1973; Kaeckenbeeck, 1979 cited in Rijkhoff et al., 1997b), but no further reports exist to our knowledge.

2.3.4 Stimulation of sacral roots and nerves

Sacral root stimulation

The first electrical stimulation technique to develop into a commercially available device for bladder emptying in cord injured people began with the work of Brindley (1977) as well as that of Tanagho and Schmidt's group (Heine et al., 1977; Schmidt et al., 1979). These investigators reported positive results using a sacral anterior root stimulator to elicit voiding in spinalized animals. This was followed by successful outcomes in humans with spinal cord injury (Brindley et al., 1982; Tanagho et al., 1989). Brindley's device was commercialized as the Finetech-Brindley Bladder System (Finetech Medical Ltd., Welwyn Garden City, UK) and has been implanted in over 2,500 people, in some cases for over 20 years (Rijkhoff, 2004b). This system has been described and reviewed in detail in a number of articles, so only a summary of the device will be presented here (Brindley, 1977; Brindley et al., 1982, 1986; Creasey, 1993; Egon et al., 1998).

The two prerequisites for implantation of sacral anterior root stimulators are intact parasympathetic preganglionic neurons and a detrusor that is able to contract (Creasey, 1993). Electrodes can either be implanted intradurally (see Figure 2.2H) on the S2-S4 anterior roots (Brindley et al., 1982) or extradurally (see Figure 2.2I) on the mixed sacral roots within the spinal canal (Lee, 1997; Sauerwein et al., 1990). In either case, the procedure is usually combined with sacral posterior rhizotomy to abolish hyperreflexive bladder and sphincter contractions, autonomic dysreflexia triggered by bladder fullness and pain in patients with incomplete lesions (Brindley, 1994; Creasey, 1993). After electrode placement, leads are tunneled subcutaneously to an implantable receiver, activated by an external controller through a radiofrequency link (Brindley et al., 1982). Figure 2.5 shows the components of this system. After implantation and posterior rhizotomy, the majority of patients are continent, have increased bladder capacity, are able to void using their stimulator with residual volumes <30 mL and are freed from catheter usage leading to a great reduction in urinary tract infec-

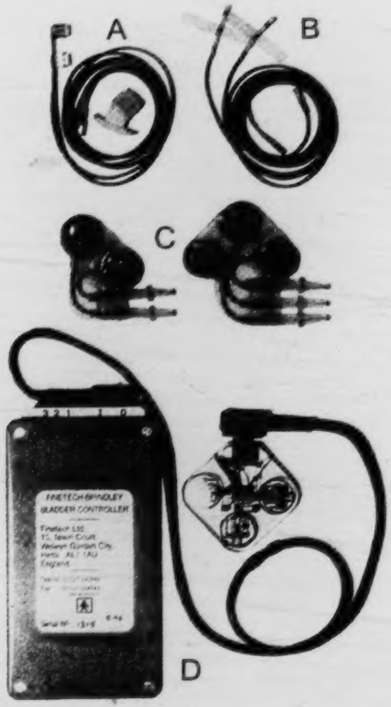

Figure 2.5: *The components of the Finetech-Brindley sacral anterior root stimulator*

(A) Intradural electrodes and leads. (B) Extradural electrodes and leads. (C) 2 and 3 channel implantable receiver blocks. These components are implanted subcutaneously and connect to the electrode leads. (D) The external components of the device including the stimulator and transmission block that is placed on the skin over the receiver block. Adapted from Egon et al. (1998).

tions (Van Kerrebroeck et al., 1993). Additionally, patients have reported beneficial stimulator-driven erections and defecation (Brindley et al., 1986; Egon et al., 1998; Van Kerrebroeck et al., 1993).

One limitation of this device is that electrical stimulation of the sacral roots, in addition to producing sustained increases in bladder pressure, activates the external urethral sphincter due to the presence of both small diameter parasympathetic preganglionic fibers and large diameter somatic fibers in the sacral anterior roots (Brindley, 1977). Since large fibers have lower thresholds of electrical stimulation, excitation of the parasympathetic preganglionic fibers is accompanied by excitation of the somatic fibers, leading to external urethral sphincter contraction and urethral occlusion. The Finetech-Brindley sacral anterior root stimulator circumvents this problem by utilizing the differ-

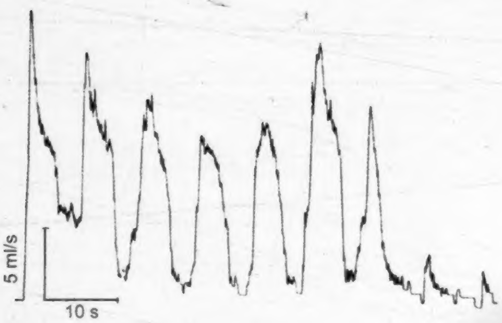

Figure 2.6: *Urine flow rate in a subject using the post-stimulus voiding technique*

During stimulation, the flow rate drops to nearly zero as contraction of the sphincter occludes the urethra. Reprinted with permission from Brindley et al. (1982).

ence in the relaxation time of the detrusor and the sphincter (Brindley et al., 1982). A train of electrical stimuli is applied for 3-9 seconds, allowing bladder pressure to rise behind the closed sphincter. Upon cessation of stimulation, the striated sphincter relaxes quickly while bladder pressure is transiently maintained allowing post-stimulus voiding (Brindley et al., 1982) (see Figure 2.6). Despite the supranormal bladder pressures that occur with this technique, no evidence of vesicoureteric reflux or hydronephrosis has been found (Creasey, 1993). Sacral roots also contain fibers innervating the musculature of the legs, and leg movement during stimulation can be cumbersome to some patients.

Given the proven benefits to people and the large number of other neuroprosthetic devices implanted in patients (Rijkhoff, 2004b), one might ask why more Finetech-Brindley sacral anterior root stimulators have not been implanted. This is likely due in part to the unwillingness of people to undergo the irreversible posterior rhizotomy, which, in addition to its very great benefits, abolishes reflex erection, defecation and micturition as well as any remaining perineal sensation. Implantation of this system is also technically demanding and attempts to market the device in the United States as the Vocare^{TM} Bladder System by NeuroControl Corp. (Cleveland, OH, USA) were ultimately unsuccessful for commercial and regulatory reasons (Hall, 2003), despite evidence that long term use could realize a reduction in costs for management of lower urinary tract dysfunction (Creasey and Dahlberg, 2001). Commercialization in the USA has recommenced through NDI Medical (Cleveland, OH, USA).

Sacral nerve neuromodulation

In the early 1980's, Tanagho and Schmidt began implanting extradural sacral root stimulators in patients (Tanagho and Schmidt, 1988; Tanagho et al., 1989). It was found that continence could be controlled by low-frequency, low-amplitude stimulation to maintain sphincter contraction without concomitant detrusor contraction (Tanagho and Schmidt, 1988). As contractions of the detrusor are inhibited by contractions of the sphincter, it was also noted that stimulation causing sphincter contraction could inhibit detrusor activity leading to improvements in continence in a range of neurogenic and non-neurogenic bladder conditions (Tanagho and Schmidt, 1988; Tanagho et al., 1989). This neuromodulatory aspect of these implants was pursued and techniques for accessing the sacral nerves though the sacral foramina were developed (Schmidt et al., 1990) making the implant procedure faster and less invasive than spinal implantation of extradural electrodes. Since this time, sacral nerve neuromodulation for treating non-neurogenic bladder dysfunction including incontinence, urgency-frequency and urinary retention have been well studied (Bosch and Groen, 2000; Siegel et al., 2000). A sacral nerve stimulator based on this work has been commercialized by Medtronic as the InterStim® (Medtronic, Minneapolis, MN, USA) and implanted in more than 10,000 people (Rijkhoff, 2004b). The InterStim consists of a battery-powered implantable stimulator connected to a single quadripolar electrode usually inserted through the S3 sacral foramen to lie next to the S3 spinal nerve (see Figure 2.2K and Figure 2.7). Sacral neuromodulation is only effective in a subset of patients with the above-mentioned bladder dysfunctions, so all patients are initially evaluated with a percutaneous electrode connected to an external stimulator to assess their response to this treatment before permanent implantation (Bosch and Groen, 2000; Siegel et al., 2000). Other reviews provide more detail about this technology and its history (Groen and Bosch, 2001; Jezernik et al., 2002; Middleton and Keast, 2004; Rijkhoff, 2004b; Schmidt, 1988; van Balken et al., 2004; Van Kerrebroeck, 2002).

With the effectiveness of sacral nerve neuromodulation, a number of investigators have examined this modality for treating neurogenic detrusor overactivity in small-scale studies of subjects with spinal cord injury. Improvements in incontinence and increases in maximal cystometric capac-

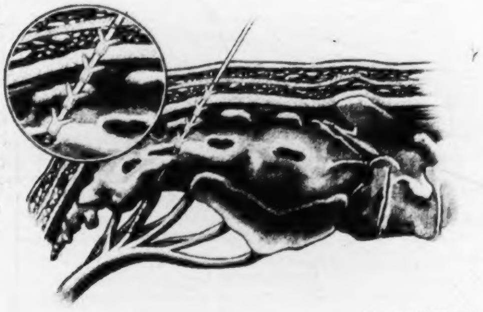

Figure 2.7: *Sacral root neuromodulation implant*

Insertion of the quadripolar electrode through the S3 sacral foramen to lie next to the S3 nerve. This electrode insertion can be performed percutaneously to test the acute response of a patient to neuromodulation. Reprinted with the permission of Medtronic, Inc. ©2005.

ity have been demonstrated in subjects with incomplete spinal cord injury (Chartier-Kastler et al., 2001; Hohenfellner et al., 2001; Ishigooka et al., 1998). However, S3 sacral nerve neuromodulation in subjects with complete spinal cord injury has been generally less effective (Chartier-Kastler et al., 2001) or had no effect at all (Hohenfellner et al., 2001; Schurch et al., 2003) leading to the suggestion that intact spinobulbospinal pathways contribute to the success of sacral neuromodulation (Schurch et al., 2003). On the other hand, stimulation of the mixed S2 root extradurally using the Finetech-Brindley stimulator without posterior rhizotomy, has successfully suppressed hyperreflexive bladder contractions in people with complete spinal cord injury and neurogenic detrusor overactivity (Kirkham et al., 2002). The differing results observed using the S2 and S3 spinal nerves for neuromodulation in subjects with complete spinal cord injury may indicate a fundamental difference in the neural pathways being excited in these two cases: namely that S3 neuromodulation has a larger supraspinal component than S2 neuromodulation.

These results suggest that the success of neuromodulatory techniques for people with spinal cord injury may depend on the completeness of the injury as well as the specific location of the electrodes. Given the commercial availability of the InterStim and the straightforward and well-established evaluation techniques, sacral neuromodulation will likely continue to be investigated for its usefulness in treating cord-injured people with neurogenic detrusor overactivity.

NeoPraxis Praxis/Minax

In 1983, a multi-channel implantable neuroprosthesis targeting sacral roots and nerves, based upon existing cochlear stimulation technology from Cochlear Ltd. (Lane Cove, NSW, Australia), was proposed (reviewed in Davis et al., 2001). After successful implantation of a 22 channel system for standing (Davis et al., 1997, 1994) a functional electrical stimulator (FES 24-A; NeoPraxis Pty. Ltd., Lane Cove, NSW, Australia) that included electrodes intended to restore bladder function was implanted in a thoracic (T)10 paraplegic (Davis et al., 1999). Three pairs of electrodes intended to elicit bladder contractions were inserted bilaterally through the sacral foramina targeting the S2-S4 spinal nerves. An epidural electrode, intended to suppress detrusor hyperreflexia, was implanted on the conus medullaris to obviate the need for dorsal rhizotomies (Davis et al., 2001). Increases in bladder pressure and some voiding was reported with intermittent stimulation, but few data were presented (Davis et al., 1999, 2001). Two additional subjects were implanted with similar systems, but bladder contractions could not be elicited (Benda et al., 2003; Smith et al., 2002). However, in these subjects, external urethral sphincter activity caused by low-frequency stimulation of the sacral nerves was reduced by selective high-frequency blockade of the large somatic fibers (Benda et al., 2003; Shaker et al., 1998). A conference report introduced the Minax system, a subset of the Praxis FES 24 stimulator specifically for bladder control (Houdayer et al., 2002), but no further reports have been published on either the Minax or Praxis systems and the company NeoPraxis appears to have become inactive.

2.3.5 Stimulation of the spinal cord

During the late 1960's and early 1970's, experiments were performed by Nashold and Friedman in animals (Nashold et al., 1971) and humans (Nashold et al., 1972) to test the efficacy of deep stimulation of the spinal cord (see Figure 2.2L) to restore micturition after spinal cord injury. These experiments were conducted based on earlier studies demonstrating that electrical stimulation of the cut sacral spinal cord could elicit bladder contractions (Stewart, 1899). It was proposed that electrical stimulation of the presumed sacral micturition center (Kuru, 1965) would be capable of causing coordinated micturition after spinal cord injury. Two electrodes, forming a bipolar pair, were implanted in the intermediolateral gray matter of the sacral spinal cord (Friedman et al., 1972; Nashold et al., 1971). Optimal rostrocaudal electrode placement was determined by monitoring the change in bladder pressure during stimulation of the dorsal surface of the sacral spinal cord. The location causing the greatest increase in bladder pressure was selected as the location for implantation of the penetrating electrodes. Friedman et al. (1972) reported that 6 of 11 animals with intact spinal cords and 5 of 9 animals with transected spinal cords voided during acute experimentation. Additionally, in chronic experiments, stimulation in 5 of 6 animals with intact spinal cords and 5 of 10 animals with transected spinal cords produced voiding. In animals where voiding did not occur, bladder pressures elicited by electrical stimulation were low, possibly indicating poor electrode placement. While some reduction in pelvic floor electromyogram, suggesting coordinated sphincter inhibition, was occasionally noted, stimulus spread resulting in activation of the sphincter was also observed (Friedman et al., 1972). Studies conducted by another group, utilizing a variety of electrode designs and stimulation parameters, concluded that sphincter motoneurons in the spinal cord were always stimulated with the sacral parasympathetic nucleus but that post-stimulus voiding could be used successfully to empty the bladder (Jonas et al., 1975; Jonas and Tanagho, 1975).

Based on these results, 27 patients, 17 in the USA, 9 in France and one in Sweden, were implanted with penetrating spinal cord electrodes (see Figure 2.8) beginning in 1970 (Nashold et al., 1981). These patients are believed to be the only people in the world implanted with electrodes targeting intraspinal structures. The implanted device consisted of two electrodes, connected to a subcutaneous radiofrequency receiver that could be activated by a handheld stimulator placed over the skin. The report on the initial four patients (3 male, 1 female) with electrodes implanted at the S1 level eventually showed good voiding in three patients (Nashold et al., 1972). Unlike some of the animal work, electrical stimulation did not generally cause concomitant relaxation of the urethra in these patients. Rather, a spastic external urethral sphincter prevented micturition in the male subjects even though large increases in bladder pressure were achieved. To overcome this, partial transurethral sphincterotomies were performed that allowed voiding in two of the male patients without causing incontinence. The female subject was able to void with an intact sphincter. This general pattern was reported in most

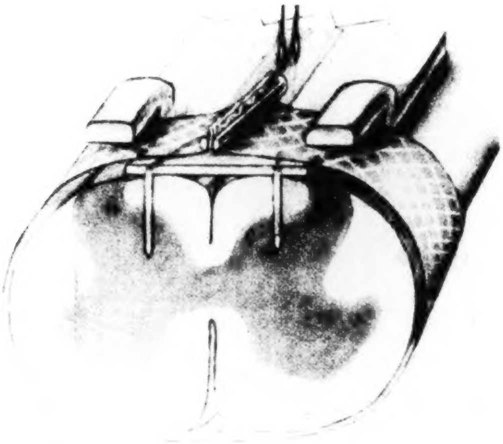

Figure 2.8: *Diagram of Nashold's intraspinal implant*

Diagram showing the location of the penetrating electrodes implanted into the spinal cord. Reprinted with permission from Nashold et al. (1972).

patients in a 10-year review of the technique (Nashold et al., 1981). Good voiding was achieved in 10 of 13 females subjects, but in only 5 of 14 male subjects. Clinical results from Duke University, where the technique was developed, reported success in 6 of 7 female subjects and 4 of 7 male subjects (Nashold et al., 1981). Of the male subjects, two voided successfully after bladder neck resections or partial sphincterotomies but two others failed to void even after these procedures were performed. However, two male subjects did achieve concomitant relaxation of the urethra leading to complete bladder evacuation without sphincterotomy, suggesting that at least in some patients, spinal cord stimulation can elicit coordinated voiding (Grimes et al., 1975). Ultimately, 60% of the subjects obtained clinically good micturition with low residual volumes, reductions in urinary tract infections, increases in bladder capacity and freedom from catheterization. Reductions in spasticity, as well as erections in some male patients, and defecation in some female patients were reported. However, autonomic and motor responses including sweating and lower limb movement often accompanied stimulation of the spinal cord (Nashold et al., 1981).

Despite these reasonably good clinical results, no further implants using this procedure were performed. There were several reasons for the abandonment of this approach. One reason is that the procedure, which involves highly invasive surgery, was unsuccessful in 40% of the subjects (Nashold et al., 1981) presumably due to ineffective electrode placement. Since neither coordinated micturition nor selective stimulation of the bladder were achieved in most patients, this procedure offered no advantages over Brindley's sacral anterior root stimulator system (Brindley et al., 1982) but added the complication of a more unpredictable electrode placement compared to sacral root electrode implantation. The inability to achieve stimulation of the bladder without concomitant sphincter activity was likely due to stimulus spread and the close proximity of the sacral parasympathetic nucleus and Onuf's nucleus (de Araujo et al., 1982; Kuru, 1965).

2.4 Future devices for electrical control of the bladder

2.4.1 Modifications of sacral root stimulators

The Finetech-Brindley sacral anterior root stimulator has proven to be the only commercially successful electrical stimulation device to restore voiding in people with spinal cord injury. Despite its proven efficacy (Brindley, 1994; Van Kerrebroeck et al., 1993) and low risk of complications and technical failures (Brindley, 1995), two issues exist, that if overcome, could help improve the function and acceptance of this device. The posterior rhizotomy that is performed in conjunction with the implantation of the Finetech-Brindley sacral anterior root stimulator eliminates neurogenic detrusor overactivity, detrusor-sphincter dyssynergia and autonomic dysreflexia triggered by bladder afferents, but irreversibly eliminates reflex erections, reflex defecation, reflex micturition and any remaining perineal sensation. The second issue is that both detrusor and sphincter efferent fibers are activated during stimulation, resulting in the functional, but non-physiological, post-stimulus voiding pattern associated with this implant. Rather than abandoning stimulation of sacral roots because of these limitations however, several techniques are being developed to address these issues. Three promising techniques are discussed here. Additional techniques to reduce or eliminate stimulation-induced sphincter contractions are reviewed by Rijkhoff et al. (1997b).

Sacral posterior and anterior root stimulation

One of the primary benefits of posterior rhizotomy in spinal cord injury is the abolition of neurogenic detrusor overactivity allowing low-pressure storage of urine and an increase in bladder capacity. Since inhibition of reflexive bladder contractions has been demonstrated in some people with spinal cord injury using sacral nerve neuromodulation techniques (Chartier-Kastler et al., 2001; Hohenfellner et al., 2001; Ishigooka et al., 1998), neuromodulation of sacral roots was investigated in 5 subjects with complete spinal cord injury and neurogenic detrusor overactivity who underwent implantation of a Finetech-Brindley system without the usual posterior rhizotomy (Kirkham et al., 2002). Electrodes were implanted extradurally on the mixed sacral roots in 4 subjects and intradurally on anterior, posterior (see Figure 2.2J) and mixed roots in the remaining subject. Since posterior rhizotomy was not performed, this system was referred to as a "Sacral Posterior and Anterior Root Stimulator" (Kirkham et al., 2002). In the three subjects that exhibited neurogenic detrusor overactivity after implantation, stimulation with small pulse widths successfully inhibited hyperreflexive bladder contractions and increased bladder capacity to a level similar to that obtained with anticholinergic medication. However, intermittent stimulation at larger pulse widths to induce voiding was unsuccessful, in spite of large increases in bladder pressure, because of detrusor-sphincter dyssynergia between stimulation periods. Less than 50% of the bladder volume was voided.

The sacral posterior and anterior root stimulator system is an important next step in the development of sacral root stimulation in that neurogenic detrusor overactivity, previously abolished by posterior rhizotomy can be eliminated by posterior root neuromodulation. However, detrusor-sphincter dyssynergia, also previously abolished by posterior rhizotomy, prevents bladder voiding in this system. Clinical use of the sacral posterior and anterior root stimulator system will require additional techniques to inhibit detrusor-sphincter dyssynergia without posterior rhizotomy to allow effective voiding.

Selective anodal block

Anodal blocking offers a method to block action potential propagation in large fibers, effectively allowing selective stimulation of the small parasympathetic fibers innervating the bladder. Selective stimulation of these fibers would reduce the concomitant contraction of the sphincter and lower limbs that presently occurs with the Finetech-Brindley sacral anterior root stimulator. This could restore a more physiological voiding pattern. Anodal blocking takes advantage of the fact that axons are hyperpolarized under the anode, reducing their excitability. Since larger diameter axons have a lower threshold for electrical activation, they can be selectively hyperpolarized (Accornero et al., 1977; Rijkhoff et al., 1994a). Stimulation at the cathode excites both large somatic fibers and the smaller parasympathetic fibers, but action potential propagation in the somatic fibers is blocked at the hyperpolarized portion of membrane, allowing selective transmission in the parasympathetic fibers (see Figure 2.9). Brindley and Craggs (1980) successfully tested this technique in animals to achieve selective parasympathetic fiber stimulation with a sacral anterior root stimulator, but it did not work well enough in humans for regular use (Brindley et al., 1982). More recently, anodal blocking has been examined in modeling studies to determine stimulation parameters (Fang and Mortimer, 1991; Rijkhoff et al., 1994a). Additionally, both animal studies (Fang and Mortimer, 1991; Grunewald et al., 1998; Koldewijn et al., 1994; Rijkhoff et al., 1994b) and intraoperative humans studies (Rijkhoff et al., 1997a, 1998) of anodal blocking have demonstrated large decreases in sphincter and leg activity while producing bladder contractions.

Several issues remain with anodal blocking however. Anodal blocking waveforms being examined currently are monophasic and require pulse widths of approximately 600 μs in humans (Rijkhoff et al., 1998). Depending on the currents required, long duration pulses can lead to irreversible electrochemical reactions at the electrodes and eventual nerve damage (McCreery et al., 1990). However, methods to increase safety by reducing the charge per phase used to achieve anodal block are being examined (Vuckovic and Rijkhoff, 2004). Additionally, implantable stimulators capable of producing the waveforms generally required for anodal blocking do not exist, although they are under development (Bugbee et al., 2001; Rijkhoff, 2004a). Although anodal blocking allows selective activation

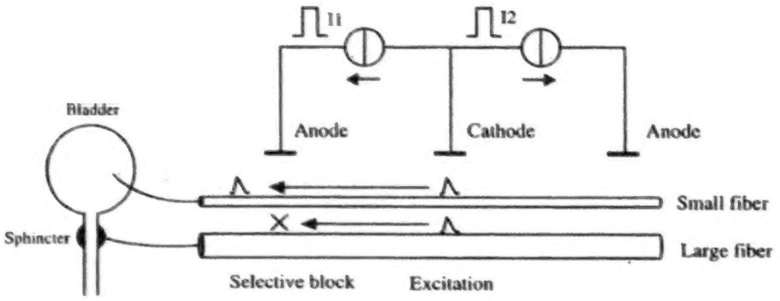

Figure 2.9: *Anodal blocking*

Electrical current under the anode hyperpolarizes the membrane of large fibers at lower stimulation amplitudes than smaller fibers. This section of hyperpolarized membrane blocks the transmission of action potentials generated at the cathode preventing activation of the sphincter while allowing activation of the bladder. Action potentials propagate and are blocked in the large fibers in both directions. The tripolar cuff design minimizes current spread and I1 and I2 are the two independent current sources sharing a common cathode. Reprinted with permission from Rijkhoff et al. (1997a).

of the bladder, posterior rhizotomies may still be required to allow voiding. In one animal study, anodal blocking without posterior rhizotomy never resulted in voiding because of reflexes that increased intraurethral pressure. In the same experiment however, continuous voiding was achieved once a posterior rhizotomy was performed (Grunewald et al., 1998). In another animal study, complete voiding was achieved using anodal blocking without posterior rhizotomy (Koldewijn et al., 1994). Even if posterior rhizotomy is required to allow voiding when using anodal blocking, this technique may allow a more physiological continuous voiding pattern and may reduce unwanted leg movements that currently occur with sacral anterior root stimulators.

High-frequency blockade

Another technique to prevent external urethral sphincter activation with sacral anterior root stimulators uses high-frequency stimulation to block action potential propagation in somatic fibers. Sawan, Elhilali and colleagues have successfully stimulated mixed sacral roots with high-frequency (600 Hz), low-amplitude pulses to block urethral sphincter efferent activity while superimposing low-frequency, high-amplitude pulses to activate parasympathetic preganglionic efferents that in turn generate bladder contractions (Abdel-Gawad et al., 2001; Shaker et al., 1998) (see Figure 2.10). High-frequency stimulation has been shown to block action potential propagation by hyperpolarizing axons and maintaining them in their refractory period (Solomonow et al., 1983). High-frequency, low-amplitude stimulation hyperpolarizes large fibers but the stimulation amplitude is not high enough to affect the smaller parasympathetic fibers. In a study of 12 chronically implanted dogs, all voided with <20% residual urine and 7 of the group voided with <10% residual urine (Abdel-Gawad et al., 2001). This system represents a potential advantage over the current Finetech-Brindley sacral anterior root stimulator where the bladder and sphincter contract simultaneously and voiding occurs post-stimulus.

This device, including a proposed method of neuromodulation to inhibit the hyperreflexive bladder by stimulation of the sacral nerves, has been patented (Sawan and Elhilali, 2002). While this group has not published results regarding the proposed neuromodulatory action of their device, proof of principle has been demonstrated in cord-injured people with a similar device (Kirkham et al., 2002). Neuromodulation could remove the necessity for posterior rhizotomy in sacral root stimulators, especially since successful voiding without posterior rhizotomy has been achieved with this stimulation paradigm, at least in animals (Abdel-Gawad et al., 2001). A brief report from another group using the same stimulation paradigm suggests that it is also effective in humans (Benda et al., 2003). Although no reports in humans exist from Sawan and Elhilali's group, Victhom Human Bionics (Saint-Augustin-de-Desmaures, Quebec, Canada) has licensed the technology and is continuing development of the device.

2.4.2 Intraspinal microstimulation

Since Nashold and Friedman's original work on spinal cord stimulation to evoke micturition (Nashold et al., 1971, 1972), interest has persisted in this technique. In these experiments, electrodes were on the order of 0.3 - 0.4 mm in diameter with 0.5 - 1.0 mm long exposed tips (Jonas et al., 1975; Nashold et al., 1971) leading to geometric electrode surface areas of 0.5 - 1.4 mm^2. Stimulus spread from these comparatively large electrodes between the adjacent sacral parasympathetic nucleus and Onuf's nu-

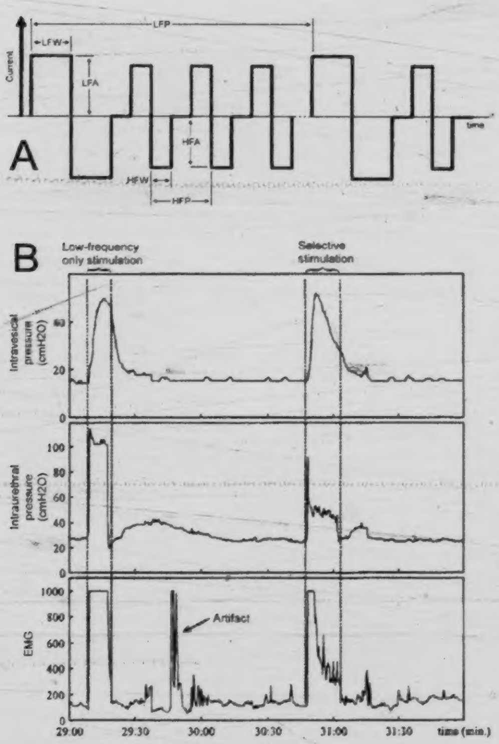

Figure 2.10: *High-frequency blockade of large sacral root fibers*

High-frequency block of large fibers allowing selective activation of the bladder. Low-frequency pulses lead to activation of large and small fibers, while high-frequency pulses block action potential propagation in large fibers selectively. (A) High-frequency, low-amplitude pulses superimposed on low-frequency, high-amplitude pulses. LFA - low-frequency amplitude, LFP - low-frequency period, LFW - low-frequency pulse width, HFA - high-frequency amplitude, HFP - high-frequency period, HFW - high-frequency pulse width. (B) Difference between low-frequency only stimulation and combined low- and high-frequency stimulation. Intraurethral pressure and sphincter EMG were reduced while bladder pressure was maintained when the selective stimulation (high-frequency, low-amplitude) waveform was utilized. Reprinted from Boyer et al. (2000) with permission from the IEEE.

cleus (de Araujo et al., 1982; Kuru, 1965) was the likely cause of observed concomitant bladder and sphincter contractions (Nashold et al., 1972). Electrode development has improved the ability to selectively stimulate specific regions within the spinal cord (McCreery et al., 2004; Mushahwar et al., 2000; Prochazka et al., 1976). Currently, electrode arrays for chronic intraspinal microstimulation use microwire electrodes (see Figure 2.11A) or silicon substrate microelectrodes manufactured using photolithographic processes (see 2.11B). Microwire-based electrodes use iridium and platinum-iridium alloy wires 20 – 30 μm in diameter with 20 - 100 μm long exposed tips resulting in geometric electrode surface areas of 1,600 - 10,000 μm^2 (Mushahwar et al., 2000). Silicon substrate microelectrodes used for chronic intraspinal microstimulation implants can have multiple stimulation sites at various depths per penetrating shank with electrode surface areas around 2000 μm^2 (McCreery et al., 2004). These electrode surface areas are 50 - 300 times smaller than the smallest electrodes used in the first spinal cord stimulation experiments (Jonas et al., 1975; Nashold et al., 1971) and allow selective stimulation of the sacral parasympathetic nucleus without concomitant sphincter activation (Carter et al., 1995; Grill et al., 1999). Although electrode arrays can cause inflammatory reactions, glial scarring and neural death around the implantation site, this can be minimized (McCreery et al., 2004) and would presumably be just as safe or safer in long term use as the larger electrodes implanted in humans (Nashold et al., 1981).

If intraspinal microstimulation is to be clinically useful, clear advantages over sacral root stimulation must be offered, especially since an intraspinal microstimulation implant would likely be at least as difficult to perform as a sacral anterior root stimulator implant. One advantage of intraspinal microstimulation is that bladder contractions can be evoked without concomitant sphincter contractions (Carter et al., 1995; Grill et al., 1999). However, more importantly, intraspinal microstimulation allows the possibility of activating sacral interneuronal networks that produce coordinated micturition or some part thereof (Grill, 2000; Grimes et al., 1975; Nashold et al., 1971).

Networks of interneurons responding to pelvic and pudendal afferents and receiving projections from the pontine micturition center exist in various regions around the central canal, in the dorsal gray commissure and in the intermediolateral cell column of the sacral spinal cord (reviewed in de Groat et al., 1996; Shefchyk, 2001) and are active during micturition (Buss and Shefchyk, 2003;

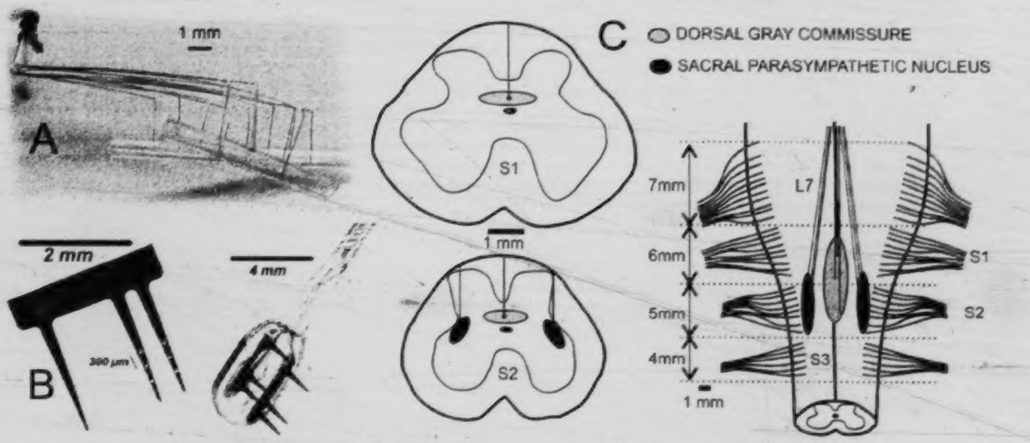

Figure 2.11: *Intraspinal microstimulation of the sacral cord*

Electrode designs and target locations (in the cat) for intraspinal microstimulation. (A) Microwire electrode array with electrodes tips placed in protective tubing. (B) Multi-site penetrating silicon electrode array. (C) Electrode targets in the cat sacral spinal cord. Electrical stimulation in the dorsal gray commissure, which contains interneurons with inhibitory projections to sphincter motoneurons, can elicit relaxation of the external urethral sphincter. Electrical stimulation of sacral parasympathetic nucleus, which contains bladder preganglionic neurons, can elicit sustained increases in bladder pressure. Adapted from Prochazka et al. (2002a) and McCreery et al. (2004).

Grill et al., 1998). One group of interneurons, located in the dorsal gray commissure, are of particular interest in relation to inhibition of the external urethral sphincter as the interneurons contain inhibitory neurotransmitter, receive direct projections from the pontine micturition center and are believed to project to Onuf's nucleus (Blok et al., 1997; Sie et al., 2001). Since Onuf's nucleus does not receive inhibitory projections from supraspinal centers, inhibitory interneurons in the dorsal gray commissure may mediate voluntary relaxation of the sphincter (Blok, 2002). This view is supported by the finding that electrical stimulation in the dorsal gray commissure produced active and sustained decreases in urethral pressure in spinally intact cats (Blok et al., 1998; McCreery et al., 2004). Additionally, some voiding can occur when electrodes around the central canal are stimulated (Grill et al., 1999). The ability to actively inhibit urethral activity is not currently possible with sacral root stimulation. These results demonstrate the potential of ISMS to achieve coordinated micturition through bladder excitation and sphincter inhibition.

Current research on intraspinal microstimulation is therefore focused on simultaneous stimulation of the sacral parasympathetic nucleus to produce bladder contractions and the dorsal gray commissure to actively relax the urethra (see Figure 2.11C). This approach has produced sustained high-pressure bladder contractions, coordinated increases in bladder pressure and decreases in urethral pressure, and occasional incomplete voiding (Prochazka et al., 2003b), but so far it has proven unreliable. One possible reason, is that in addition to the interneurons in the dorsal gray commissure that inhibit sphincter motoneurons, there are other interneurons in this same region have been shown to decrease their firing rate during micturition, and may be part of pathways with excitatory connections to sphincter motoneurons (Buss and Shefchyk, 2003). If this is the case, electrical stimulation in parts of the dorsal gray commissure may, in fact, activate more excitatory interneurons that inhibitory interneurons, and thereby cause contraction, rather than relaxation of the external urethral sphincter. It is also unknown whether intraspinal microstimulation can affect the activity of the smooth muscle internal urethral sphincter, which is the primary mechanism to maintain continence until the bladder is very full or at high pressure. These results were obtained in an awake animal, suggesting that previous results in anesthetized animals may also hold true in the absence of anesthesia. Additionally, electrodes targeting the sacral parasympathetic nucleus produced similar increases in bladder pressure using the same stimulation parameters, before and after complete spinal cord transection. However, bladder pressure increases produced by stimulation in the sacral parasympathetic nucleus and urethral pressure decreases produced by stimulation in the dorsal gray commissure may not be sufficient to produce micturition. In some cases, simultaneous intraurethral pressure recordings in the vicinity of the external urethral sphincter and bladder pressure recordings, have indicated that voiding should occur (Prochazka et al., 2003a) (see Figure 2.12), but once the urethral pressure catheter was removed to unblock the urethra, stimulation through the same electrodes did not elicited voiding. In this case, high pressure in the bladder neck or distal urethra may have prevented voiding.

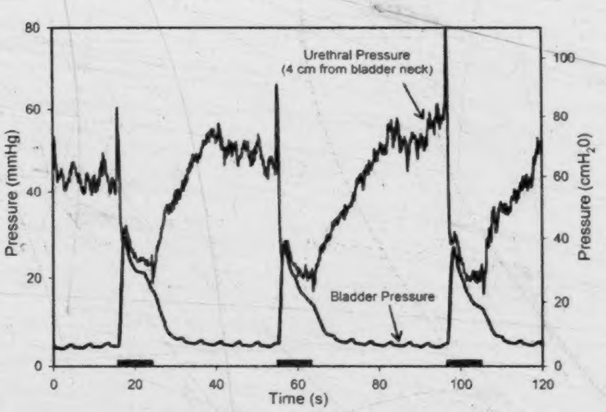

Figure 2.12: *Bladder and urethral pressure changes in response to ISMS*

Bladder and urethral pressure changes in response to electrical stimulation through three electrodes: two targeting the sacral parasympathetic nucleus and one targeting the dorsal gray commissure. During stimulation, immediate increases in bladder pressure and decreases in urethral pressure were achieved. The pressures measured in the bladder and urethra became essentially equal (within the calibration error of the transducers) during stimulation indicating that voiding might occur in the absence of the urethral catheter. However, once the catheter was removed to unblock the urethra, stimulation through the same electrodes did not induce voiding. Adapted from Prochazka et al. (2003a).

2.4.3 Urethral afferent stimulation

Electrical stimulation of afferent branches of the pudendal nerve, specifically the dorsal penile nerve, has been shown to inhibit hyperreflexive bladder contractions occurring after spinal cord injury. However, electrical stimulation of urethral afferents, also forming part of the pudendal nerve, has been shown to elicit bladder contractions as well as relaxation of the sphincter. This spinal reflex was first described by Barrington in spinal cord-transected cats (Barrington, 1914, 1941), and more recently have been investigated in cord-transected cats (Gustafson et al., 2003; Shefchyk and Buss, 1998) and humans with spinal cord injury (Gustafson et al., 2003, 2004). These reflexes are presumed to facilitate voiding by positive feedback from afferents sensitive to urethral dilation (Shafik et al., 2003a,b). In humans, urethral afferents were electrically stimulated using a catheter-mounted electrode (Gustafson et al., 2003) passed into the urethra (see Figure 2.2M). In subjects with complete spinal cord injury, bladder contractions reaching 70 cmH$_2$O as well as voiding could be achieved if the bladder volume was above a threshold value (Gustafson et al., 2004). Below this threshold, bladder contractions could not be generated and voiding was therefore incomplete. Despite the fact that only incomplete voiding has been demonstrated, this stimulation technique is very intriguing from the perspective of neuroprosthesis device development. So far, it is the only way in which bladder contractions can be generated without invasive implantations of electrodes targeting the bladder, sacral roots or spinal cord itself. However, even more interesting is the apparent ability of urethral afferent stimulation to evoke coordinated micturition after complete spinal cord injury (Shefchyk and Buss, 1998).

2.4.4 Microstimulators

Implanted neuroprostheses discussed in this review have all used the same basic set of components. An implanted stimulator, either battery powered or inductively coupled to an external power source, is implanted subcutaneously in a convenient location such as the abdominal or chest region and long leads connect the electrodes to the stimulator. This design can lead to time-consuming surgical procedures as well as technical failures including lead breakage and connector failure (Brindley, 1995). Microstimulators such as the BION^{TM} (Advanced Bionics, Valencia, CA) provide an alternative approach. BIONs are self-contained, injectable microstimulators that are programmed and powered by inductive coupling. A single external transmitter coil can communicate with up to 256 BIONs, each of which can deliver current controlled pulses with a pulse width range of 4 – 512 μs and amplitude range of 0 – 30 mA up to frequencies of 50 pulses per second in a package 2 mm in diameter and 16 mm long (Loeb et al., 2001) (see Figure 2.13). A more recent version of the BION includes a lithium-ion battery to power the device during normal use, but is programmed and recharged using the external coil. This device is larger (3.3 mm diameter, 27 mm long) (Groen et al., 2004), but allows subjects to be free of the external coil and associated hardware during daily activities, at least in applications not requiring phasic control.

Two studies have evaluated the use of BIONs for treating bladder dysfunction using the inductively powered (Buller et al., 2002) and battery powered (Groen et al., 2004) systems. Although final

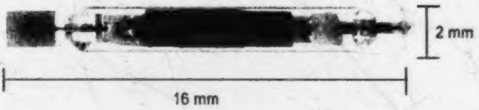

Figure 2.13: *A BION^{TM}*

Photograph of a glass encapsulated BION. Adapted from Loeb et al. (2001).

reports have yet to be published, both studies have shown positive results using pudendal nerve (see Figure 2.2N) stimulation to treat overactive bladder incontinence. BIONs have not been clinically tested in spinal cord injury patients for bladder control, but a number of the stimulation techniques presented in this review could be adapted to use BIONs.

2.4.5 MiniatURO

The miniatURO (Biocontrol Medical Ltd., Yahud, Israel) is a closed-loop implantable electrical stimulation system for treating mixed urinary incontinence. The device consists of an intra-abdominal pressure sensor, pelvic floor stimulation electrode and a stimulator with integrated control system. Stimulation is triggered by increases in intra-abdominal pressure. This device has been tested in short-term settings where abdominal pressure was measured rectally, stimulating electrodes were introduced percutaneously, and the stimulator was worn externally. All subjects in a group of 16 with stress incontinence responded positively and became dry or had reduced episodes of incontinence (Nissenkorn et al., 2004). While this device has not been tested in cord-injured people, there is reason to believe that this kind of device might be effective in the spinal cord injury population (Fjorback et al., 2003).

2.4.6 Transcutaneous magnetic stimulation

Transcutaneous magnetic stimulation of the nervous system is a noninvasive method of activating neural tissue and has therefore become a useful technique for human experimentation and clinical diagnostics. Two studies have examined the ability of magnetic stimulation to exert control over lower urinary tract function in spinal cord injury subjects but have arrived at very different results

(Bycroft et al., 2004; Lin et al., 1997). In the first study it was concluded that repetitive magnetic stimulation (15 – 30 Hz) of the sacral nerves, achieved by placing the stimulation coil over the sacrum (see Figure 2.20), caused direct activation of parasympathetic preganglionic neurons innervating the bladder leading to increases in bladder pressure. Fatigue of the external urethral sphincter and intermittent stimulation to allow post-stimulus voiding were the two methods postulated to explain the voiding that occurred, even though many subjects had received sphincterotomies (Lin et al., 1997). The more recent study suggests that magnetic stimulation of the sacral nerves causes direct inhibition of bladder contractions in cord-injured people with neurogenic detrusor overactivity. It also stated that the previously described bladder contractions were rebounds occurring at the end of stimulation as a result of releasing the detrusor from direct inhibition (Bycroft et al., 2004).

Whatever the effect of magnetic stimulation on bladder function in cord-injured people, this technique holds promise for a device-based therapy for bladder control. Current magnetic stimulation systems are large and would not be suitable for chronic use. However, the potential benefits of this completely noninvasive technique, and its demonstrated efficacy for treating neurogenic detrusor overactivity and/or eliciting partial voiding, deserve further investigation.

2.5 Successes and failures of devices for bladder control

A wide variety of techniques and devices have been developed to manage the significant problems associated with lower urinary tract dysfunction after spinal cord injury. While devices such as the Finetech-Brindley sacral anterior root stimulator can truly be said to exert control over the lower urinary tract, other devices, including catheters merely manage the symptoms associated with lower urinary tract dysfunction after spinal cord injury. However, the ultimate goal of any treatment modality, including pharmacological and surgical methods, is to create a bladder capable of storing large volumes of urine at low pressure, while preventing incontinent episodes and allowing periodic evacuation of that urine at low pressure. If a treatment achieves its intended function, then the merits of one technique versus another depend on issues such as adverse side effects, procedural reversibility, device cost, ease of use and ease of implantation.

2.5.1 Device efficacy, advantages and disadvantages

Two classes of devices have been described in this review: mechanical devices and electrical stimulation devices. The mechanical devices discussed range from the very simple to the very complex and address voiding dysfunction, incontinence and detrusor-sphincter dyssynergia. A brief summary of the efficacy, main advantages and disadvantages, and current status of these devices is presented in Table 2.1. Several general comments about the use of mechanical devices can be made. Catheters and the In-Flow intraurethral pump do not require implantation. They are also commercially available, allowing easy adoption by clinicians. Artificial urethral sphincters and urethral stents do require surgery, but this is relatively simple. However, none of these devices offer the potential to restore lower urinary tract function to the pre-injury state. There are also significant side effects due to the chronic presence of foreign materials in the lower urinary tract. Finally, no mechanical device is able to affect neurogenic detrusor overactivity, so pharmacological or surgical treatments must be used to establish a high-volume, low-pressure bladder.

Electrical stimulation devices, while not curative, offer the unique potential to restore normal function to the lower urinary tract after spinal cord injury. Many techniques have been presented in this review that vary significantly in their ability to establish a high-volume, low-pressure bladder and to produce low-pressure voiding. Improving continence, storage volume and storage pressure by inhibiting hyperreflexive bladder contractions has been the specific aim of a number of devices. A brief summary of the efficacy, main advantages and disadvantages, and current status of these devices is presented in Table 2.2, while a similar summary for devices intended to evoke bladder emptying is presented in Table 2.3. Of the electrical stimulation techniques intended to inhibit hyperreflexive bladder contractions, both dorsal penile nerve stimulation and tibial nerve stimulation have shown promising results. However, these methods require additional study with the use of more practical devices that are suitable for chronic use. Bladder wall, pelvic nerve and spinal cord stimulation are all methods to elicit voiding have been tested in humans with spinal cord injury and subsequently abandoned. However, bladder wall stimulation and spinal cord stimulation continue to be explored in the laboratory setting.

Table 2.1: *Summary of mechanical devices used for lower urinary tract management after spinal cord injury*

Device	Efficacy	Advantages	Disadvantages	Current Status
Catheter	Very good	Simple Inexpensive	Urinary tract infections Inconvenient	Clinical
Artificial sphincter	Mixed	Proven design	Infection Urethral erosion	Limited use
Urethral stent	Good	Reversible alternative to sphincterotomy	Not for long-term implantation	Some clinical
Intraurethral pump	Good	Simple implant	Regular replacement Discomfort	Investigational

Table 2.2: *Summary of electrical stimulation devices used to control continence after spinal cord injury*

Device	Efficacy	Advantages	Disadvantages	Current Status
Pelvic floor maximal functional electrical stimulation	Mixed	Long-lasting improvements	Physical and psychological discomfort Inconsistent results	Limited use
Thigh stimulation	Mixed	Non-invasive Long-lasting improvements	No predictors for success Time consuming treatment	Limited use
Dorsal penile nerve surface stimulation	Promising	Non-invasive	Not well studied	Investigational
Tibial nerve stimulation	Promising	Simple treatment procedure	Permanent device required Not well studied	Investigational
Sacral neuromodulation	Mixed	Minimally-invasive Proven design and implant procedure Commercially available	May not work in complete injuries Inconsistent results Not well studied	Investigational

Table 2.3: *Summary of electrical stimulation devices used to control voiding after spinal cord injury*

Device	Efficacy	Advantages	Disadvantages	Current Status
Intravesical electrical stimulation	Mixed	Long-lasting results Can improve voluntary voiding in incomplete injuries	Ineffective in many patients No predictors for success Time consuming treatment	Limited use
Bladder wall stimulation	Mixed	Simple surgical approach	Sphincter and pelvic floor contraction Electrode-bladder interface failure	Abandoned except for lower motoneuron lesions
Pelvic nerve stimulation	Poor	–	Sphincter and pelvic floor contraction Difficult surgical approach Nerve unsuitable for stimulation	Abandoned
Sacral root stimulation	Very good	Voiding without catheterization Low residual volume Proven design and implant procedure Long term usage with few failures Commercially available	Posterior rhizotomy required Demanding surgical implant Unphysiological voiding	Clinical
Spinal cord stimulation	Good	Voiding without catheterization Low residual volume Long-term usage	Sphincter contraction Sphincterotomy required in males Difficult electrode placement 40% failure rate	Originally abandoned Currently investigational

2.5.2 Clinical success of devices in managing dysfunction after spinal cord injury

Catheters and urethral stents, as well as dorsal penile nerve, tibial nerve, spinal cord and sacral root stimulation have all been shown to be effective in managing various aspects of lower urinary tract dysfunction after spinal cord injury. However, the success of these devices from a clinical perspective varies significantly. The clinical success of catheters remains foremost. Of the other effective techniques, only the Finetech-Brindley sacral anterior root stimulator could be considered a proven clinical success with over 2,500 systems implanted (Rijkhoff, 2004b). This system is successful because it is the only device that reliably evokes complete bladder evacuation, significantly reducing the complications associated with catheterization. When combined with sacral posterior rhizotomy, as it nearly always is, the Finetech-Brindley sacral anterior root stimulator provides a complete system for lower urinary tract control after spinal cord injury. However, the consequences of irreversible rhizotomy, the technically demanding implant procedure and commercial and regulatory issues have limited the availability of this device as well as the acceptance of it by patients and clinicians. With this in mind, given the large number of people that could benefit from such a device, and a generally increasing clinical acceptance of neuroprostheses in general, the Finetech-Brindley sacral anterior root stimulator has had a limited impact on the spinal cord injury population throughout most of the world.

2.5.3 The future of devices for bladder control

Progress is being made on regeneration of the spinal cord, but a complete biological cure for spinal cord injury is unlikely to be developed in the near future (Fawcett, 2002). In light of this, and the fact that eventual regenerative therapies will likely be combined with neuroprostheses to maximize functional recovery (Prochazka et al., 2002b), new and improved devices are required to restore control of the lower urinary tract after spinal cord injury. The feasibility and efficacy of a number of mechanical and electrical devices for treating lower urinary tract dysfunction has been clearly demonstrated in spinal cord injury patients. However, no clinical device can be said to have solved the problem of bladder control as low-pressure physiological voiding can not yet be produced and no device has successfully incorporated methods to produce both voiding and suppression of neurogenic detrusor overactivity. While future improved biomaterials will undoubtedly reduce some of the side effects associated with the use of mechanical devices (Beiko et al., 2004), these are unlikely to achieve complete control over the lower urinary tract. Electrical stimulation devices on the other hand have demonstrated the ability to achieve significant control over neurogenic detrusor overactivity and voiding. Adaptations of the Finetech-Brindley sacral anterior root stimulator, including posterior root stimulation and anodal blocking or high frequency blockade of somatic fibers, offer the possibility of inhibiting hyperreflexive bladder contractions and producing physiological voiding without the currently requisite posterior rhizotomy. Combining such methods may significantly improve future electrical stimulation devices for bladder control.

Other electrical stimulation techniques currently under investigation also show promise. Intraspinal microstimulation has the potential to utilize remaining spinal cord networks and can achieve selective stimulation of the bladder as well as active inhibition of the sphincter, albeit not reliably enough to produce consistent voiding. Intraspinal microstimulation research has yet to address the problem of neurogenic detrusor overactivity and surgical implantation of intraspinal microstimulation devices in their current form would be even more difficult than implantation of sacral root stimulators. Unless simpler electrode configurations are developed, this may limit clinical use of intraspinal microstimulation even if it can be shown to be reliable and effective in animals. Stimulation of the dorsal penile nerve and urethral afferents are among the most interesting techniques currently being investigated to restore control of the lower urinary tract from a neuroprosthetic device development perspective. They offer the potential of a minimally invasive implant to suppress neurogenic detrusor overactivity and produce voiding, perhaps using microstimulators. If these techniques are to have an impact on the clinical management of lower urinary tract dysfunction after spinal cord injury, they must provide clear improvements in treatment over what can be currently accomplished with catheters, pharmacological therapies and surgical intervention.

An ideal device for controlling lower urinary tract function after spinal cord injury can be postulated based on the material presented in this review. Above all, the device must suppress neurogenic detrusor overactivity and allow user-controlled, continuous, low-pressure voiding with a low residual volume. The device should not require additional pharmacological or surgical procedures to operate successfully and would ideally require only minimally invasive surgery. Although not discussed in this review, an implant to restore lower urinary tract function should ultimately provide means to restore bowel and sex function as well. Finally, any procedure must be completely reversible so that people are not committed to a particular device for the rest of their lives and are able to take advantage of future devices.

2.6 Conclusions

In this review we have discussed devices and techniques aimed at restoring normal function to the lower urinary tract after spinal cord injury. Some of these devices and techniques have ultimately proven to be unsuccessful for reasons such as insufficient efficacy, unacceptable treatment procedures, side effects and technical failures. Other devices, most notably catheters and sacral root stimulation, have been successful. Over 20 years of experience with sacral root stimulation to restore voiding clearly indicates the advantages that people with spinal cord injury can expect to achieve with neuroprostheses. However, detrusor-sphincter dyssynergia must be overcome before neuroprostheses can gain more widespread use. Electrical stimulation techniques to suppress neurogenic detrusor overactivity, allowing efficient bladder filling and storage, have been demonstrated in some cord-injured people. But relatively few studies have been conducted and many details regarding these techniques have yet to be elucidated. These, or other similar techniques, must be successful if a neuroprosthesis for complete control of lower urinary tract function is to be developed. Perhaps the most difficult and crucial problem is achieving relaxation of the sphincter to allow physiological voiding. It has been said that "The key to control of the bladder lies in control of the sphincter" (Schmidt, 1986). While this comment was originally made in reference to the ability of sphincter activity to modulate bladder contractility, it is equally true that the ability to control the sphincter and eliminate unwanted activity remains the final piece of the puzzle that must be put in place for bladder control neuroprostheses to be completely effective. A number of techniques are being investigated to accomplish this, but no single one has completely achieved this goal. In conclusion, while many devices and techniques have been tried and ultimately abandoned, the successes clearly show the immense benefits that can be achieved with the use of devices in the control of the dysfunctional bladder.

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Chapter 3

Viability of intraspinal microstimulation as a neuroprosthetic technique for bladder control

"The brain is like a muscle. When it is in use we feel very good. Understanding is joyous."

– Carl Sagan

3.1 Introduction

After spinal cord injury (SCI), one of the more critical and enduring medical issues is the management of lower urinary tract (LUT) function. Before this was fully appreciated, the primary cause of mortality after SCI was renal failure caused by ureteric reflux as a result of a full bladder. Since the introduction of clean intermittent catheterization (Guttmann and Frankel, 1966; Lapides et al., 1972) the number of cases of renal failure has greatly declined (Frankel et al., 1998), but complications involving the LUT, especially urinary tract infections, are still the most common cause of rehospitalization after SCI (Middleton et al., 2004).

Many electrical stimulation devices to manage LUT dysfunction have been developed (Gaunt and Prochazka, 2006), the most successful of these being sacral anterior root stimulators (Brindley, 1977; Brindley et al., 1982). More than 2,500 people have been implanted with this system, some for over 20 years (Rijkhoff, 2004). As a result of the neuroanatomy of the sacral roots, stimulation results in co-contraction of the bladder and sphincter. To overcome this, stimulation is applied in 3-9 second trains interrupted by periods of no stimulation to allow the sphincter to relax. The sphincter, being mainly skeletal muscle, relaxes quickly while pressure in the bladder, which is smooth muscle, drops slowly. Voiding occurs during these relaxation periods resulting in so called "post-stimulus voiding" (Brindley et al., 1982). Posterior rhizotomies are usually performed during the implantation to eliminate hyperreflexive activity of the bladder and sphincter, which in turn improves bladder capacity, reduces incontinence and eliminates post-stimulus reflex sphincter activity. Unfortunately, rhizotomies are irreversible and have other consequences such as a permanent loss of sexual reflexes and remaining perineal sensation (Brindley, 1994; Creasey, 1993), making this procedure unattractive to certain patients. These devices are also only readily available to people in a few European locations. Though sacral anterior root stimulation is very effective in those people with SCI who opt for it, there remains a strong motivation to develop improved methods to manage LUT dysfunction after SCI (Anderson, 2004).

One alternative to stimulation of the sacral roots is to stimulate directly within the spinal cord to activate the desired neuronal pools selectively. Stimulation of the spinal cord using penetrating electrodes was first examined as a means to improve bladder function in the pioneering work of Drs. Nashold and Friedman in the 1960's and 1970's (Nashold et al., 1971, 1972). This work eventually led to the implantation of bipolar penetrating electrodes in the sacral spinal cords of 27 patients worldwide (Nashold et al., 1981). Contrary to some of the preliminary tests in animals, intraspinal stimulation frequently resulted in concomitant bladder and sphincter contractions, causing some of the 27 patients to require sphincterotomies to achieve good voiding. Ultimately 60% of the patients achieved good clinical results although undesirable autonomic and somatic motor responses were frequently elicited (Nashold et al., 1981). These procedures were discontinued after a few years but they did demonstrate the feasibility of intraspinal stimulation as a technique. A more detailed review of this history is provided by Gaunt and Prochazka (2006).

Recently, intraspinal microstimulation (ISMS) has been investigated as a means to restore standing and stepping after SCI (Lau et al., 2007; Mushahwar et al., 2000, 2002; Saigal et al., 2004). ISMS involves the implantation of arrays of small electrodes within the spinal cord, targeting specific populations of neurons. Studies investigating ISMS for bladder control have shown that bladder contractions can be achieved by microstimulation within the sacral parasympathetic nucleus (SPN) without concomitant sphincter contractions (Carter et al., 1995; Grill et al., 1999). Sphincter relaxation has also been demonstrated by stimulation within the dorsal gray commissure (DGC) of the sacral spinal cord (Blok et al., 1998; Carter et al., 1995) presumably through GABAergic inhibition of sphincter motoneurons (Blok et al., 1997). A recent study has reported near-complete voiding in a small percentage of chronically implanted (2-14 months), lightly anesthetized cats using stimulation through microwire arrays implanted in the sacral cord (Pikov et al., 2007). From these previous studies, it is reasonable to hypothesize that stimulation within the SPN and DGC in conscious animals with and without SCI would elicit bladder contractions and sphincter relaxation resulting in micturition.

3.2 Materials and Methods

To investigate whether or not ISMS is a feasible approach for restoring micturition after SCI, arrays of microwires were implanted in the sacral spinal cords of ten adult male cats (weight: 2.7 – 5.3 kg). Specifically, electrodes were implanted targeting the SPN and DGC to elicit bladder contractions and sphincter relaxation, respectively. Nearly all testing sessions were conducted in awake animals to allow the results to be directly applicable to any future clinical work. All experiments were done with the approval of the University of Alberta Animal Care and Use Committee.

3.2.1 Implant fabrication

Electrode arrays consisting of 12 to 16 platinum-iridium microwires were implanted in each animal. The wires were 25 μm to 30 μm in diameter and were insulated with polyimide. The last 50 – 100 μm of each wire was deinsulated and sharpened by cutting at an acute angle. The ends of each wire were bent at 90° so that the deinsulated tip reached the targeted intraspinal structure. Typically electrodes targeting the DGC were 2.5 mm in length and electrodes targeting the SPN were 3 to 3.5 mm in length. Since the electrodes were implanted through the dura mater, the subdural space (~1 mm) was added to the depth of the DGC (~1.5 mm) and the SPN (~2 to 2.5 mm) from the cord dorsum. The microwires were fixed together to locate each of the electrodes in the appropriate rostro-caudal locations. The wires were typically arranged in three columns: the central column containing the electrodes targeting the midline DGC and the two lateral columns containing electrodes targeting the SPN bilaterally. Figure 3.1(A and B) shows a schematic of the ISMS implant and the approximate locations of the SPN and DGC within the sacral spinal cord. The exact number, length and inter-electrode spacing varied slightly from implant to implant. The wires were fed through a Silastic tube and coiled to allow the tube to stretch without damaging the wires. Each wire was soldered to a pin on a headpiece connector. The solder joints were insulated with 3-5 coats of aerosol-based conformal coating (Loctite 3900) and the back of the connector was potted in epoxy (Loctite E-30CL Hysol) to provide mechanical stability. In addition to the microwires, a length of stainless steel wire (Cooner AS632) was included in the implant to act as the indifferent electrode. A two centimeter length of wire was deinsulated and positioned subcutaneously near the lumbar spine.

A bladder catheter suitable for long-term implantation was manufactured using Silastic tubing (2.16 mm OD, 1.02 mm ID). Plastic molds were machined to fit around the tubing to form a retaining flange and to plug the end of the tube. RTV 108 was used to form the flange and plug. The retaining flange had a diameter of 6 mm, was approximately 1 mm thick and was located 11 mm from the end of the tube. Holes approximately 1.5 mm in diameter were cut on either side of the tubing near the tip. Figure 3.1C shows a schematic of the catheter end.

Two requirements were met with the implants described above. First, we felt it important to be able to insert each electrode individually to varying depths, the whole array being distributed over about a 15mm rostro-caudal length of the spinal cord. Electrodes mounted on a rigid substrate, as used for example by Pikov et al. (2007) have a fixed spatial relationship and a relatively small area of coverage, that may not be optimal in a given implant. Having individual wires allowed customized placement guided by intra-operative stimulation. Second, the bladder catheter was developed to allow long-term access to the bladder. This allowed experiments to be conducted without any anes-

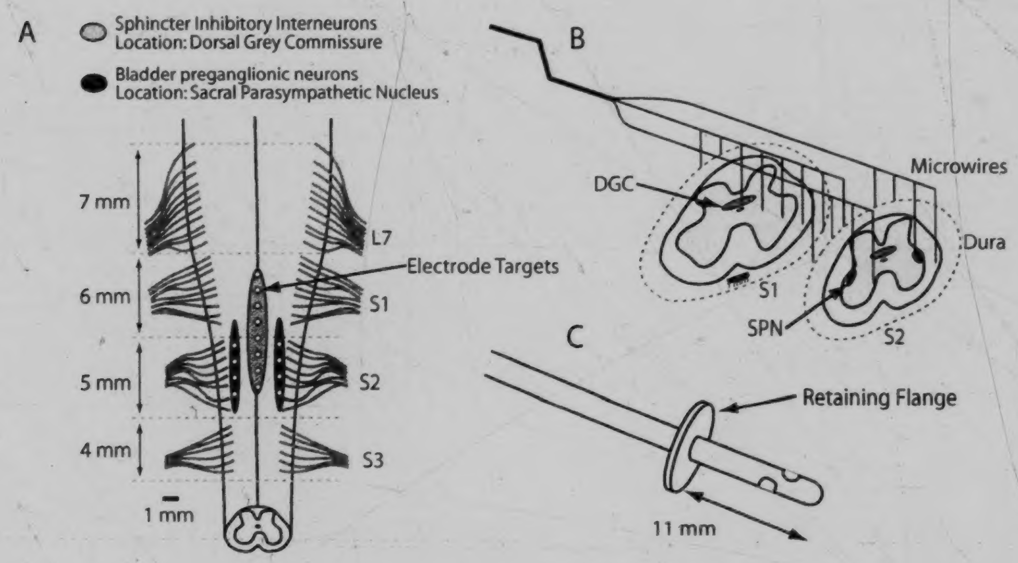
A
Sphincter Inhibitory Interneurons
Location: Dorsal Grey Commissure
Bladder preganglionic neurons
Location: Sacral Parasympathetic Nucleus
7 mm
6 mm
5 mm
4 mm
1 mm
L7
S1
S2
S3
Electrode Targets
B
DGC
Microwires
Dura
S1
SPN
S2
C
Retaining Flange
11 mm

Figure 3.1: *Schematic representation of the implant*

(A) Dorsal view of the spinal cord show spinal segments, locations of targeted motoneurons pools and representative locations for ISMS electrodes (open circles). (B) Schematic view of ISMS implant showing microwires penetrating through the dura mater into target gray matter structures. (C) Schematic of chronically implanted bladder catheter.

thesia, since an intraurethral catheter did not need to be placed to add or withdraw fluid from the bladder or to measure bladder pressure.

3.2.2 Surgical implant procedures

Surgeries were performed in a fully-equipped operating room with sterile equipment and procedures. The cats were anesthetized with ketamine (25 mg/kg im) and intubated using a pediatric tracheal tube. Pre-operative medication was administered: acepromazine (0.25 mg/kg im), atropine (0.04 mg/kg im) and buprenorphine (0.01 mg/kg sc). Anesthesia was maintained with 2-3% isoflurane in carbogen at 1.5 L/min. An intravenous catheter was inserted in the cephalic vein and a saline drip was delivered throughout the procedure. Body temperature was maintained using a warm-water heating pad. Respiration and heart rate were monitored throughout the procedure which typically lasted 6-8 hours.

The abdominal cavity was opened through a midline incision and the bladder was exposed. The bladder was drained with a "tomcat" catheter (Kendall, 3.5 Fr., Mansfield, MA) and refilled with 10 mL sterile saline. A purse-string suture was placed in the dome of the bladder using a 4-0 silk suture which was initially left untied. A 16 gauge needle was then used to puncture the bladder and the resulting hole was held open using fine forceps while the bladder catheter was inserted up to the retaining flange. The purse-string suture was then tied and four additional sutures were used to secure the flange on the bladder catheter to the bladder wall. The abdominal muscles were sutured closed with 3-0 Vicryl or catgut suture, the catheter emerging at the rostral end of the incision. The skin was later sutured closed after the catheter had been passed subcutaneously to the headpiece (see below).

A laminectomy was performed to remove the L6 lamina and spinous process and caudal portion of the L5 lamina. A 30 μm diameter stainless steel "search" electrode 3.5 mm long was inserted through the dura mater into the spinal cord to locate the best spot to elicit bladder contractions. Once the wire had been inserted, the spinal cord was stimulated using current-controlled 200 μs pulses, ranging from 30 to 150 μA in amplitude at 50 pulses per second. During stimulation, intravesical pressure was measured and displayed on an oscilloscope. Details of the pressure recording technique are described in a following section. Potential stimulation sites in the sacral spinal cord were explored with repeated insertions of the search electrode to different depths. The search volume typically encompassed three or four spinal segments, up to 2.5 mm lateral to the midline on both sides and depths of up to 4 mm from the dural surface. This technique of searching was thought to provide the most reliable way of identifying the locations eliciting the strongest bladder responses. For reference, the SPN extends up to 10 mm rostro-caudally with the majority of cells located in the S2 spinal segment (Nadelhaft et al., 1980). In the transverse plane, the SPN forms an oval shaped nucleus with a major diameter of 1 mm and a minor diameter of 200 μm oriented along the lateral edge of the intermediate region of the gray mater (Nadelhaft et al., 1980). Less is known about the functionally relevant extent of the DGC, however one of the original experiments reported stimulation induced reductions in intraurethral pressure from caudal L7 to S3 over dorso-ventral distances of 1 mm (Blok et al., 1998). Once the location providing the largest increase in intravesical pressure was determined, the microwire array was positioned so that the central bladder electrodes were at the same rostro-caudal location as the search electrode. The silicone tubing containing the wire loom was attached to the L5 spinous process with dental acrylic and the wires were implanted into the spinal cord through the dura mater. During implantation, each electrode targeting the SPN was inserted to a depth at which stimulation through that electrode elicited a maximal amplitude bladder contraction. Electrodes targeting the DGC were inserted into the midline of the cord with their rostro-caudal locations being fixed by the construction of the implant itself. No intra-operative testing of DGC electrodes was performed. Each microwire was secured to the dura mater with small droplets of cyanoacrylate glue applied with a syringe and 30G hypodermic needle whose beveled tip had been sawn off. A sheet of thin plastic film was placed on top of the wires and glued to the dura mater at the corners to minimize connective tissue growth and adhesions of overlying muscles to the dura mater. The free end of the bladder catheter and the lead of the ISMS array were tunneled subcutaneously via a trocar to the head where they were attached to a dental acrylic or machined headpiece. The bladder catheter was terminated in a standard Luer fitting. All skin incisions were closed with 3-0 Prolene suture. At extubation, the cat was given acepromazine (0.25 mg/kg sc) and buprenorphine (0.01 mg/kg sc) and one or two small doses of pentobarbital (2 mg/kg sc) sufficient to maintain a somnolent state. During post-operative recovery the cats were kept warm in heated cages and provided with blankets. Analgesia was maintained by giving two or three additional doses of buprenorphine at 8-hourly intervals. Ampicillin was administered for 4 days after surgery, followed by Amoxil (50 mg tablets, 2/day) for 6 additional days.

3.2.3 Spinalization

Spinal cord transection at T10 level was carried out in one cat (P35) approximately 6 months after the ISMS implant. Surgical preparation was the same as described above. A laminectomy was performed to create an opening at the T11-T12 vertebral junction. The dura mater was incised transversely and a solution of 2% lidocaine (0.2 mL) was dripped on the surface of the cord. Two minutes later lidocaine was injected inside the spinal cord at progressively more ventral levels. Fine scissors were used to transect the cord completely while attempting to avoid the ventral vessel. Complete transection was verified visually by retracting the proximal and distal ends of the cut spinal cord. Strips of Surgicel were placed in the gap to stop any bleeding and to maintain separation between the ends of the spinal cord. The dura mater was closed with 8-0 silk suture and a piece of thin plastic film was placed over the incision site. The wound was then closed in three layers: muscle, fascia, skin. Post-surgical care was the same as described above. After spinal transection, bladder and bowel function were continuously monitored and the bladder was emptied using the indwelling catheter at least twice daily.

3.2.4 Post-implant ISMS testing

Beginning shortly after the implantation of the microwire array, the effect of ISMS through each electrode was tested. In the majority of cases, the animals were tested in the absence of anesthesia. The animals were unrestrained for the duration of the procedure and typically rested quietly in the experimenter's lap or on a fleece rug. This was done for two major reasons. Firstly, we wanted to measure conscious sensory responses to stimulation. Approximately 50% of SCI patients have incomplete injuries (Stover, 1995), with sensory function more likely to be preserved than motor function. If ISMS elicited painful or unpleasant sensations, this would limit its potential as a clinical application. By carefully increasing stimulus intensities to levels that just started to elicit orienting reactions, we were able to address this issue without causing obvious discomfort. Secondly, we wanted to ensure that the results would be clinically relevant given that anesthetics cause changes in the excitability of the nervous system. Most anesthetics have some effect on the normal functioning of the LUT. Isoflurane at the concentrations required even for light anesthesia affects potassium and sodium channels leading to a hyperpolarizing shift in membrane potential (Duch et al., 1998; Nau, 2008) which can suppress reflex bladder contractions (Matsuura and Downie, 2000) and could reduce EUS motoneuron excitability. The anesthetic commonly used in urodynamic studies, alpha-chloralose, is not suitable for repeated chronic experimentation because of its long recovery time. Other anesthetics such as propofol are $GABA_A$ and/or glycine agonists (Trapani et al., 2000) which suppress reflexive bladder contractions (Matsuura and Downie, 2000) and can potentiate GABA currents and inhibit reuptake at the presynaptic terminal (Vanlersberghe and Camu, 2008) which could increase GABAergic inputs to EUS motoneurons originating from DGC interneurons (Blok et al., 1997). Performing most of the experiments without the influence of anesthetic was therefore felt to allow a more clinically relevant appraisal of the effects of ISMS on bladder and sphincter function.

Prior to testing each electrode, the bladder was emptied through the indwelling catheter and slowly refilled with saline. Typical bladder volumes depended on the animal being tested, but were usually in the range of 80% of the volume that triggered a voluntary void. The indwelling bladder catheter was connected via the headpiece Luer connector to a Neurolog NL108D4/10 dome and NL108T4 isolated pressure transducer (Digitimer Ltd., Welwyn Garden City UK). The pressure traces were displayed on an oscilloscope and sampled at 100 samples per second using a CED Power 1401 Laboratory Interface and Signal v3 software (Cambridge Electronic Design Ltd., Cambridge, UK). The ISMS electrodes were connected to a custom-built multi-channel microstimulator. Stimulus pulses were current-controlled, asymmetric amplitude, charge-balanced rectangular waves. The pulse rate ranged from 20-50 Hz with 50 Hz being the most commonly used frequency. When multiple electrodes were stimulated, the stimulation pulses were interleaved.

Each electrode was tested individually while monitoring changes in bladder pressure, micturition if it occurred, and any other motor or sensory responses. If changes in bladder pressure occurred, the stimulation amplitude was increased until no further increase in bladder pressure could be obtained or until sensorimotor responses became disruptive. For those electrodes where no change in bladder pressure occurred, sensorimotor thresholds were found. Combinations of electrodes eliciting bladder contractions were then stimulated together to increase the amplitude of the bladder contraction. Stimulation through electrodes targeting the EUS inhibitory DGC regions of the spinal cord were then stimulated during the bladder contraction and any voiding was measured. Since an intraurethral catheter could not be placed in the awake animal, changes in EUS activity were not directly recorded in this condition.

To more specifically investigate changes in EUS activity in response to stimulation in the DGC, a number of trials were performed with the animals anesthetized with isoflurane. Intraurethral pressure was measured using a closed-end tomcat catheter connected to a Neurolog NL108D4/10 dome and NL108T4 isolated pressure transducer. The urethral catheter was modified to block the most distal of the two side ports at the tip of the catheter. The urethral catheter was also connected to an infusion pump (Pump 22, Harvard Apparatus, Saint Laurent, Quebec, Canada) and during intraurethral pressure measurements, sterile saline was infused at a rate of 0.2 mL/min. This method of measuring urethral pressure was first described by Brown and Wickham (1969). The side port of the urethral catheter was positioned in the region of the EUS, typically 4-6 cm from the tip of the urethral meatus. The intraurethral pressure signal was displayed on an oscilloscope and sampled at a rate of 100 samples per second using a CED 1401 Laboratory Interface and Signal v3 software. The bladder pressure was recorded as previously described.

3.2.5 Data analysis

Changes in bladder pressure caused by electrical stimulation are reported as the peak pressure minus the baseline pressure unless specified otherwise. Pressures were manually read from the data files. The baseline pressure was measured during the 5 seconds prior to stimulation and the peak pressure was measured as the maximum pressure during stimulation (discounting obvious artifacts that could occur if the cat moved during the trial). Grouped data are reported as mean ± standard deviation. Comparisons of the maximum bladder contractions with and without anesthesia were done using the non-parametric Mann-Whitney U test (SigmaStat v3.5, Systat Software, San Jose , CA). Significance was set to $p < 0.05$.

At the end of each experiment, the spinal cord was carefully removed with the microwires intact and placed in fixative. The microwire locations were determined by serial sectioning of the spinal cord under a high-power surgical microscope. The microwire tip locations, and the shape of the white and gray matter were drawn.

3.3 Results

Implants were attempted in ten cats for this study. Two implants were abandoned during surgery because of an inability to locate any regions of the sacral cord that elicited bladder contractions when penetrations were made with the search electrode. A third implant failed shortly after surgery due to wire breakage. The results of the remaining seven implants are reported. The duration of these experiments were 2 to 29 weeks (median 14 weeks).

3.3.1 Intra-operative testing and positional sensitivity

During the search for the best location for the bladder electrodes during the initial surgery, peak bladder pressures were recorded in 6 animals. The peak pressure elicited across all 6 cats by the search electrode was 25.3 ± 6.1 mmHg (range: 20 – 35 mmHg). In two animals the peak pressure was not noted although intra-operative testing was carried out as normal. In two animals, no bladder contractions could be elicited intra-operatively despite extensive searching throughout the entire sacral spinal cord for several hours. Since the electrodes could be individually placed, the positions of the electrodes targeting the SPN were optimized during surgery to elicit the largest bladder contractions. However, it was noted during the search procedure and during the optimization process that very slight changes in electrode position (particularly in the dorso-ventral direction) had a large effect on the observed bladder contractions. From this observation, it was estimated that a depth change of 300 μm could result in a change from a robust bladder contraction (> 20 mmHg) to no response at all. This sensitivity was further demonstrated by the effect of securing the microwires to the dura mater with cyanoacrylate glue. As the glue set, the electrodes tended to be pulled out of the cord slightly. The difference between the peak pressure before and after gluing was measured during three implants and of 20 electrodes targeting the SPN in these implants, 14 elicited smaller contractions after gluing than before. The decrease in peak pressure was 10.2 ± 8.2 mmHg (range: 1 – 30 mmHg). It was also observed in several cats during subsequent chronic recording sessions that posture affected the evoked responses. In these animals, light pressure applied to the back of the animal also changed the ISMS-evoked responses suggesting that small movements of the ISMS electrodes were occurring.

3.3.2 Electrode locations

The post-experiment localization of electrode positions was difficult. In many cases there were large amounts of tissue ingrowth into the electrode array looms and substantial dural adhesions despite the placement of plastic film over the implants to prevent this problem. Due to these factors it was impossible to dissect tissue away from the implant site without dislodging the entire array in 4 of 7 animals. Figure 3.2 shows the electrode locations for the three animals (P17, P35, and R19) in which the arrays were not dislodged during dissection. This figure also shows the magnitude of the bladder contraction elicited by stimulation through the electrodes. The diamond-shaped symbols indicate those electrodes that were intended to target the DGC while the circular-shaped symbols indicate those electrodes that were intended to target the SPN. The distance between the final electrode position and the margin of the intended target was 0.73 ± 0.36 mm (range: 0 – 1.56 mm) with just 5 of the 37 electrodes falling within 300 μm of the target. In several cases, electrodes that missed the DGC target ended up near the SPN resulting in large increases in bladder pressure from these electrodes. The largest increases in bladder pressure (>20 mmHg) occurred through ISMS electrodes in S2 that fell within the bounds of the SPN (see arrows), in the ventral horn and in some cases in the white matter.

3.3.3 Bladder contractions

Figure 3.3 shows histograms of the maximum increases in bladder pressure obtained by ISMS through individual electrodes. The data shown were all obtained well after recovery from the implant surgery. In some cases, the animals were anesthetized to measure bladder and urethral pressures in addition to the normal awake testing. Figure 3.3 shows that many of the implanted electrodes elicited no change in bladder pressure. Of the electrodes tested in the awake condition targeting the SPN, 49% elicited an increase in bladder pressure of less than 2 mmHg while 37% elicited an increase in bladder pressure greater than 10 mmHg. Some of the electrodes targeting the DGC also elicited changes in bladder pressure. In the awake condition, 32% elicited an increase in bladder pressure of greater than 2 mmHg while 6% elicited an increase greater than 10 mmHg. Each histogram of Figure 3.3 also shows

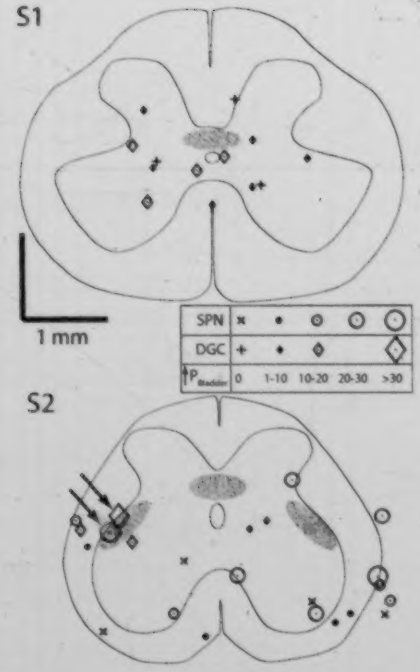

Figure 3.2: *Electrode locations*

Post-mortem electrode locations identified from three animals (P17, P35, R19). Electrode locations could not be found in the remaining animals as the electrode arrays were inadvertently dislodged from the spinal cord during dissection. This was in large part due to large dural adhesions and ingrowth of connective tissue into the implant. Electrodes targeting the SPN and DGC are separately identified by circular and diamond symbols, respectively. The size of the symbol represents the magnitude of the maximum bladder contraction elicited by the electrode during the course of the experiment in mmHg. The arrows identify the two electrodes that resulted in the largest increase in bladder pressure and are clearly within the bounds of the SPN. The shaded regions indicate the estimated bounds of the DGC (midline) and the SPN (bilateral).

the maximum increase in bladder pressure obtained from the best electrode in each animal and the mean ± SD of the maximum pressures under the given set of conditions. The mean peak increase in bladder pressure elicited by electrodes targeting the SPN in the awake cats was 20 ± 8.5 mmHg. In some cases, the animals were anesthetized to measure bladder pressure in addition to the normal awake testing. When the maximum bladder contractions elicited by each electrode under anesthesia were compared to the bladder contractions elicited while the animals were awake, no difference was found (Wilcoxon signed rank test, $p = 0.75$). Since different stimulation amplitudes were used when

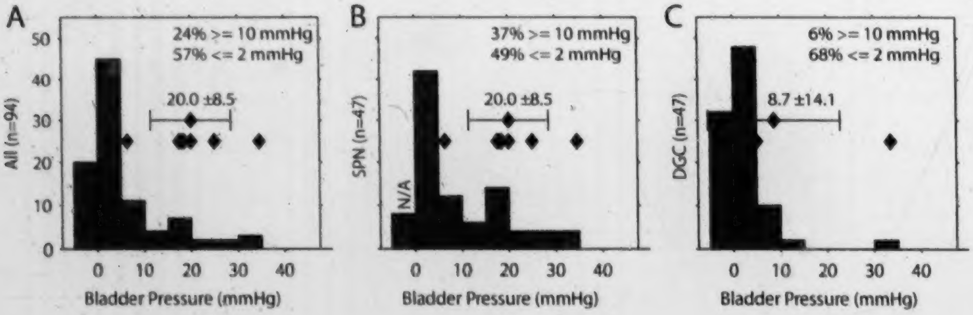

Figure 3.3: *Maximum bladder pressure histograms for all animals*

Maximum bladder pressure histograms for all animals. Bladder pressures included are the maximum pressure that was achieved using a single ISMS electrode. Each bin is 5 mmHg wide and the x-axis range in each panel is the same. The number of electrodes eliciting the indicated increase in bladder pressure is displayed on the y-axis. The total number of electrodes implanted is also shown in the y-axis labels. Untested electrodes are included in the left-most bar labeled N/A in each histogram. (A) Histogram including data from all of the implanted electrodes across all animals (n=7). (B) Histogram including data from just the electrodes targeting the SPN. (C) Histogram including data from just the electrodes targeting the SPN. In each histogram, the percentage of electrodes eliciting more than a 10 mmHg increase in bladder pressure and less than a 2 mmHg increase in bladder pressure is listed. In nearly every case, more than 50% of the total number of electrodes elicited less than a 2 mmHg increase in bladder pressure. In each histogram, the maximum bladder pressure elicited by ISMS through a single electrode in each animal is displayed as a solid diamond. The mean ± SD are also displayed for these maximal values. In the majority of cases, the best electrode in each animal elicited a bladder pressure increase of approximately 20 mmHg.

the animals were awake and when they were anesthetized, this comparison was limited to those cases where the difference in stimulation amplitude between the two conditions did not exceed 20 μA. Using a different limit did not cause the difference to become significant at any point.

It was expected that interleaved stimulation through multiple electrodes would elicit a larger bladder contraction than stimulation through a single electrode. Figure 3.4 shows two examples of the effect of interleaved stimulation on the bladder pressure. In both cases interleaving the stimulation led to larger contractions. In the first example, the increase in bladder pressure from short stimulation trains summed less than linearly, while in the second example summation was more than linear. In both cases longer trains of stimulation led to maintained bladder contractions.

In several cases, the cats voided naturally during the course of an experimental session, as a result of slow filling of the bladder with saline through the bladder catheter. Since bladder pressure was being monitored, several traces of bladder pressure during voluntary voiding were recorded. Figure

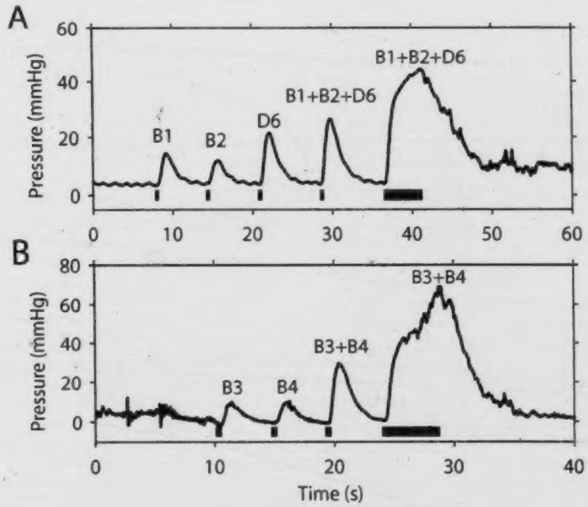

Figure 3.4: *Interleaved stimulation*

Examples of the effect of interleaved stimulation through multiple electrodes on the increase in bladder pressure in two awake cats (A – animal P35 pre-spinalization, B – animal Q3). (A) Stimulation through three electrodes individually and then interleaved. The stimulation amplitude for each electrode was 130 μA. Interleaved stimulation led to a less than linear summation of bladder pressure in the initial short bursts of stimulation, but with the final prolonged burst the bladder pressure reached 44 mmHg after about 4 seconds of continuous stimulation. (B) Stimulation through two electrodes individually and then interleaved. The stimulation amplitudes were 119 μA and 83 μA for electrodes B3 and B4, respectively. Interleaved stimulation led to more than linear summation of bladder pressure in the initial short bursts. With continuous stimulation, the bladder pressure reached 69 mmHg after 4 seconds. The black bars under each trace indicate the duration of the stimulation.

3.5 shows an example of the bladder pressure during voluntary voiding. In this example, the peak pressure during natural voiding was 53 mmHg. Similar pressures were obtained with interleaved stimulation through three electrodes, but without voiding.

Electrode stability was a major obstacle in this study. Of the electrodes that elicited an increase in bladder pressure greater than 5 mmHg during the first chronic recording session, 72% elicited no increase in bladder pressure during the last recording session. The average time between the first and last recording session was 11.5 ± 11.3 weeks.

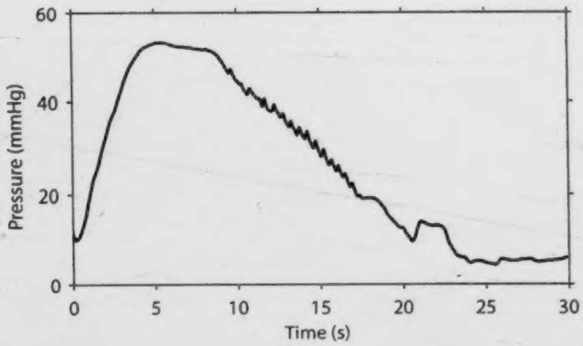

Figure 3.5: *Voluntary voiding in a conscious animal*

An example of natural voiding in one cat (P35 pre-spinalization). During a stimulation and recording session, the cat voided naturally while bladder pressure was being recorded. In this example, the peak bladder pressure reached during micturition was 53 mmHg. Natural voiding records were measured eight times in this animals and the peak pressure during voiding was 47.7 ± 5.5 mmHg. The ripples in the pressure trace as pressure declined was a characteristic feature of natural voiding.

3.3.4 Urethral relaxation

In three animals where the implant remained stable, urethral pressure responses to ISMS were investigated specifically by recording intraurethral pressure with the cats anesthetized. Figure 3.6A shows an example of urethral relaxation elicited by interleaved stimulation through five electrodes targeting the DGC. In this example, stimulation had little effect on the bladder pressure. In the three tested animals, the maximum observed reduction in urethral pressure was 25.3 ± 4.5 mmHg.

3.3.5 Micturition

Micturition was infrequently observed in this study and almost never in large quantities. Figure 3.6B shows an example where both an increase in bladder pressure and a decrease in intraurethral pressure occurred to the point where the pressures were equal (animal Q3). This equalization of pressures is the condition under which voiding would be expected to occur. However, when the intraurethral catheter was removed and the same stimulation parameters were used, no voiding occurred. Voiding was only observed in one cat (P35). Most often, this was simply several drops of urine. However, in a number of instances, streams of urine and complete, or nearly complete voiding was achieved.

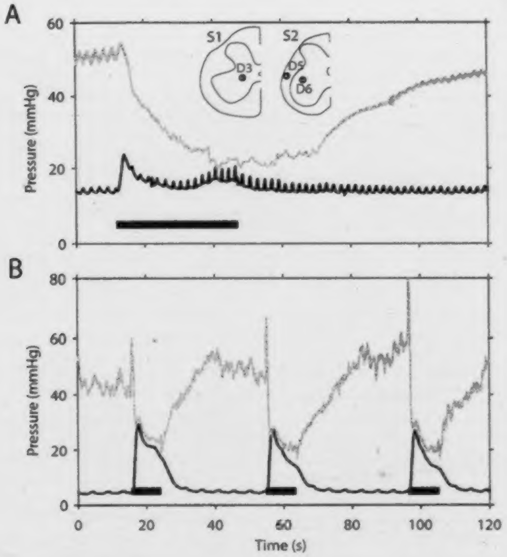

Figure 3.6: *External urethral sphincter inhibition*

Two examples of reductions in intraurethral pressure elicited by ISMS in different anesthetized animals (R19 and Q3). Intraurethral pressure traces are shown in gray and bladder pressure traces are shown in black. (A) Stimulation through five electrodes (D2 at 250 μA + D3 at 200 μA + D4 at 320 μA + D5 at 150 μA + D6 at 205 μA) targeting the DGC at 50 Hz. During the stimulation, the intraurethral pressure decreased by nearly 30 mmHg and there was virtually no change in bladder pressure. This was a very powerful stimulus, and would have likely resulted in a sensory response in the awake animal. However, it did result in a large reduction in intraurethral pressure. The known electrode locations are shown. (B) Stimulation through three ISMS electrodes (B3 at 104 μA + B4 at 150 μA + D1 at 78 μA) at 50 Hz. This combination of electrodes both elicited a bladder contraction and caused a reduction in the intraurethral pressure. During the stimulation, the pressures were isobaric indicating that the urethra was completely relaxed. However, repetition of this stimulus with the catheter removed did not result in voiding. This is likely because the bladder pressure was too low. The black bars under each trace indicate the duration of the stimulation.

In these instances, the bladder contractions outlasted the stimulation train and the cat assumed a voiding posture suggesting that the increase in bladder pressure elicited by ISMS caused the cat to void voluntarily. Of the trials where measurable voiding occurred, 31% had voiding efficiencies less than 10% and 37% had voiding efficiencies greater than 90% with all of the latter being accounted for by voluntary or triggered micturition. The animal in which some voiding did occur (P35) received a complete spinal transection at T10.

3.3.6 Spinalization

After transection, voluntary voiding was abolished as expected. Complete voiding was not triggered by ISMS either. Most frequently, we could not find any combination of ISMS through the available electrodes that was able to elicit voiding. In those rare cases in which ISMS did elicit voiding the efficiencies were never higher than 26%. Bladder contractions elicited by ISMS remained very stable in this cat before and after spinalization. Figure 3.7 shows bladder pressure changes in response to ISMS through the same three electrodes using the same stimulation parameters one day before spinal transection (Figure 3.7A), two days after transection (Figure 3.7B) and one month after transection (Figure 3.7C). Inhibition of the urethral sphincter was also achieved after spinalization. Figure 3.8 shows an example of reductions in intraurethral pressure elicited by ISMS 6 days after spinalization.

3.3.7 Sensorimotor responses

During stimulation trials, additional sensory and motor responses to ISMS were noted when they occurred. Frequently, stimulation led to hindlimb motor responses, perineal twitching, tail movements, changes in the alertness of the animal and in some cases brief vocalizations. In no cases was the stimulation increased to the point that the animal moved away or changed its resting posture. Table 3.1 lists the number of electrodes in each animal that led to unwanted motor or sensory responses. Of the electrodes targeting the SPN, 35 of 47 electrodes elicited unwanted responses and 29 of the 47 electrodes targeting the DGC elicited unwanted responses. 41% of the electrodes elicited movements of the legs while 18% of the electrodes elicited vocal responses on one or more occasions. Figure 3.9 shows the median threshold amplitudes at which these unwanted sensorimotor responses occurred in each animal for each electrode and response type. SPN electrodes elicited sensorimotor responses at a median amplitude of 60 ± 46 μA across animals, and in six animals at least one electrode elicited a sensorimotor response at less than 30 μA. Vocalizations in response to ISMS were recorded in five animals. In three of these animals, the minimum stimulation amplitude for eliciting this response was 10 μA, 12 μA and 30 μA. Across all five animals, the mean threshold for eliciting these responses was 75 ± 40 μA. However, the electrode locations for many of these electrodes are unknown.

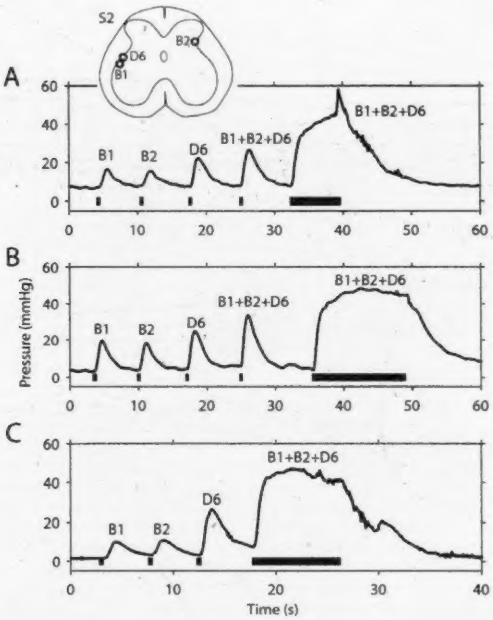

Figure 3.7: *Bladder contractions before and after spinalization*

Stability of ISMS evoked bladder contractions before and after complete spinal cord transection (animal P35). In all cases, the stimulation amplitude for each electrode was 130 µA. (A) ISMS evoked bladder contractions one day before spinalization. The locations of the electrodes are shown. (B) ISMS evoked bladder contractions 2 days after complete spinal transection at T10. (C) ISMS evoked bladder contractions 4 weeks after complete spinal cord transection. In all cases, maintained bladder contractions greater than 40 mmHg were elicited by interleaved stimulation. The labels in each plot indicate which ISMS electrodes were active. The black bars under each trace indicate the duration of the stimulation.

During stimulation trials, additional sensory and motor responses to ISMS were noted when they occurred. Frequently, stimulation led to hindlimb motor responses, perineal twitching, tail movements, changes in the alertness of the animal and in some cases brief vocalizations. In no cases was the stimulation increased to the point that the animal moved away or changed its resting posture. Table 3.1 lists the number of electrodes in each animal that led to unwanted motor or sensory responses. Of the electrodes targeting the SPN, 35 of 47 electrodes elicited unwanted responses while overall, 72 of 102 total electrodes elicited unwanted responses. 46% of the electrodes elicited movements of the legs while 19.6% of the electrodes elicited vocal responses on one or more occasions. Figure 3.9 shows the median threshold amplitudes at which these unwanted sensorimotor responses

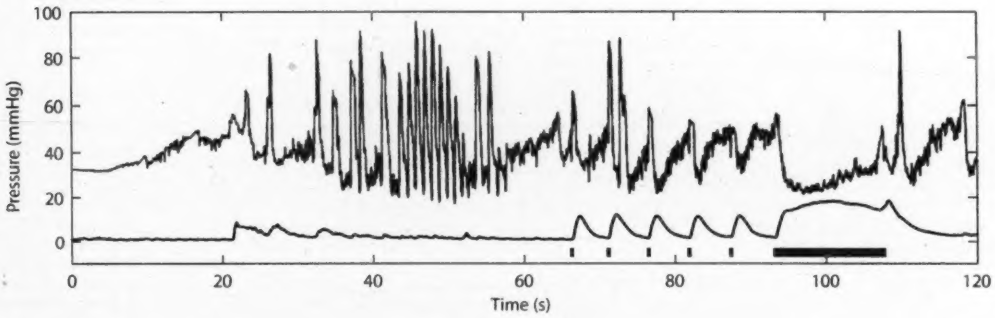

Figure 3.8: *Urethral inhibition after spinalization*

Example of urethral inhibition 6 days after complete spinal cord transection. The intraurethral pressure trace is shown in gray and the bladder pressure trace is shown in black. Spontaneous contractions of the external sphincter consistent with hyperreflexia are seen between the 20 and 60 second markers. During continuous stimulation through a single electrode at 130 μA and 50 Hz, the intraurethral pressure decreased and bladder pressure increased. The pressures were not eqibaric. The black bars under the trace indicate the duration of the stimulation.

occurred in each animal for each electrode and response type. Figure 3.9F also shows the threshold amplitudes that elicited unwanted sensorimotor responses for each electrode in the three animals where the final electrode locations were known. SPN electrodes elicited sensorimotor responses at a median amplitude of 60 ± 46 μA across animals, and in six animals at least one electrode elicited a sensorimotor response at less than 30 μA. Vocalizations in response to ISMS were recorded in five animals. In three of these animals 31%, 43% and 44% of the implanted electrodes elicited vocalizations. The electrodes with the lowest thresholds elicited these responses at 10 μA, 12 μA and 30 μA Across all five animals, the mean threshold for eliciting these responses was 73 ± 43 μA.

3.4 Discussion

In this study we investigated the feasibility of eliciting micturition in the cat by delivering ISMS through chronically implanted arrays of microwires each targeted individually to specific areas in the spinal cord. Overall the results suggest that ISMS using discrete microwires is not a suitable technique for eliciting micturition before or after SCI. While there were a number of positive results suggesting that continued effort in this area may eventually lead to a functional neuroprosthesis for

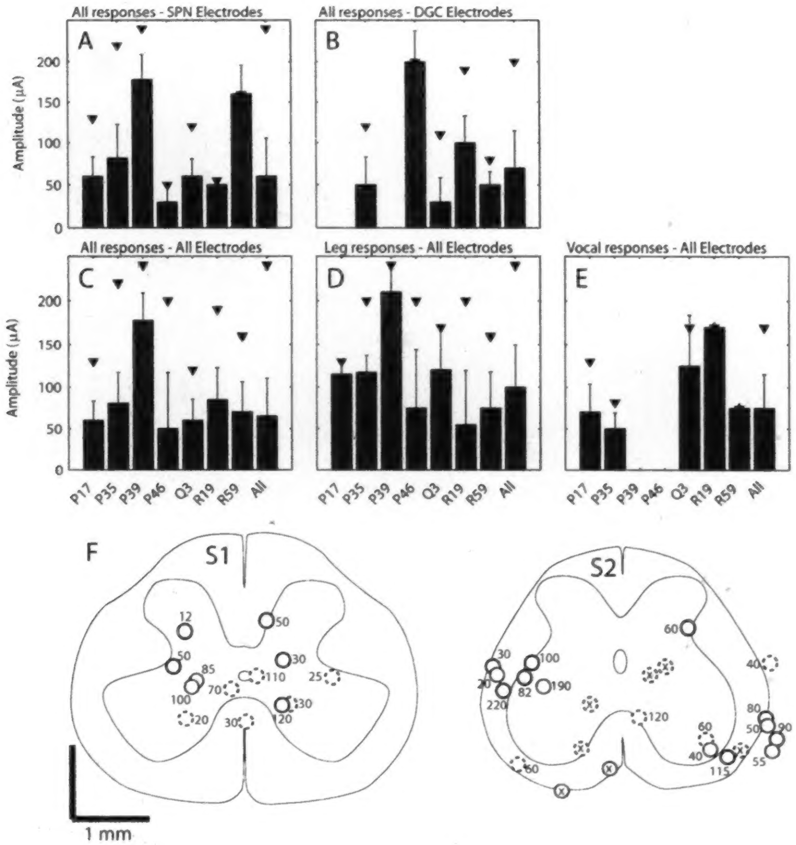

Figure 3.9: *Sensorimotor responses to ISMS*

Median of the threshold stimulation amplitudes to reach sensorimotor thresholds for each animal. Error bars are mean average deviations and the solid triangles show the maximum and minimum stimulation amplitude that elicited a sensorimotor response. In each panel the first seven columns show data for each animal while the final column is the average of the responses across all animals. (A) Median threshold for all sensorimotor responses for electrodes targeting the SPN in each animal. (B) Median threshold for all sensorimotor responses for electrodes targeting the DGC in each animal. (C) Median threshold for all responses in all electrodes for each animal. (D) Median threshold for leg movement in all electrodes for each animal. (E) Median threshold for vocalization responses for all electrodes in each animal. (F) Electrode locations in the three known cases showing the minimum stimulation amplitude that elicited an unwanted sensorimotor response on each electrode. An 'x' indicates that no response was elicited from this electrode. The dashed black circles indicate those electrodes from animal P17. The black circles indicate those electrodes from animal P35. The gray circles indicate those electrodes from animal R19. The stimulation thresholds are reported in μA.

Table 3.1: *Number of electrodes eliciting sensorimotor responses in each animal*

Summary of the number of electrodes that elicited a sensorimotor response in the awake state. Each fraction indicates the number of electrodes that elicited an unwanted response and the total number of electrodes implanted. For example in animal P17, 6 of the 8 electrodes that were implanted targeting the SPN elicited unwanted an unwanted sensorimotor response of some kind while none of the electrodes targeting the DGC elicited an unwanted response. In animal P35, 6 of 14 total electrodes implanted elicited vocalizations at some stimulation amplitude and 8 of 14 total electrodes elicited a leg movement of some kind. Overall, 64 of 94 total electrodes elicited a sensorimotor response, 39 of 94 electrodes caused a leg movement and 17 of 94 electrodes cause the cats to vocalize when stimulated. The electrode locations are known for animals P17, P35 and R19.

	All responses			Leg	Vocalization
	SPN	DGC	Total	Total	Total
P17	6/8	0/8	6/16	2/16	5/16
P35	7/7	7/7	14/14	8/14	6/14
P39	4/8	0/8	4/16	2/16	0/16
P46	7/8	4/4	11/12	6/12	0/12
Q3	5/6	6/6	11/12	9/12	4/12
R19	3/6	6/6	9/12	6/12	1/12
R59	3/4	6/8	9/12	6/12	1/12
Total	35/47	29/47	64/94	39/94	17/94

bladder control, there remain substantial obstacles to overcome before this is likely to be realized.

Since Nashold and Friedman's original work on stimulation of the spinal cord with penetrating wires to elicit micturition in cats (Friedman et al., 1972; Nashold et al., 1971) and humans (Nashold et al., 1973, 1972), there has been a considerable interest in this technique (Carter et al., 1995; Grill et al., 1999; Jonas et al., 1975; Jonas and Tanagho, 1975; McCreery et al., 2004). Earlier work frequently found evidence of concomitant bladder and sphincter activity (Jonas and Tanagho, 1975; Nashold et al., 1972) leading to difficulties in reliably achieving micturition in some of the human implantation (Nashold et al., 1981). More recent studies have shown that it is possible to activate the bladder independently of the EUS (Grill et al., 1999; Pikov et al., 2007; Tai et al., 2004) with the use of smaller electrodes. The geometric surface areas of the electrodes used in these more recent studies and also in the present study, ranged from 0.0002 to 0.01 mm^2, while the electrodes used in the earlier experiments had geometric surface areas of 0.5 to 1.4 mm2. There have also been demonstrations of reductions in intraurethral pressure elicited by ISMS in the DGC (Blok et al., 1998; Carter et al., 1995; Pikov et al., 2007). Since the DGC and SPN targets are distributed across most of the S1 and

S2 spinal segments, we felt that an electrode array capable of stimulating both targets needed to span approximately 10 mm. The microwire electrodes used in the present study were originally developed for chronic recordings in the dorsal root ganglia (Prochazka et al., 1976) and have been used recently for ISMS in the lumbar spinal cord to activate locomotor networks (Mushahwar et al., 2000; Saigal et al., 2004) and evoke standing (Lau et al., 2007).

3.4.1 Electrode position sensitivity and stability

During the search portion of the implant surgery we found that changing the depth of electrodes in the dorso-ventral direction by as little as 300 µm could substantially reduce or even eliminate the bladder contraction elicited by ISMS. This agrees well with previously published data showing similar changes in bladder contraction amplitude with changing electrode depth (Carter et al., 1995; Grill et al., 1999). It corresponds roughly to the extent of the SPN when viewed in the transverse plane (Morgan et al., 1979). However, this sensitivity is somewhat surprising given that ISMS can activate neurons through means other than direct activation of the target cell body or axon (Gaunt et al., 2006). This extreme positional sensitivity most likely explains the disappointing performance of ISMS implants in our hands. As mentioned earlier, even the slight expansion of the tissue glue used to fix the microwires to the dura mater was evidently enough in several animals to reduce the bladder contraction amplitudes elicited by ISMS. In the three cases where the final electrode positions are known, the average positional error of the electrode tips was 0.73 ± 0.36 mm which is substantially larger than what we estimate to be the allowable tolerance for successful positioning (approximately 300 µm). Just 2 of the 37 electrodes were clearly within the bounds of the SPN (see Figure 3.2).

There are several possible causes for the inaccuracy of microelectrode positions in this study. The first and most obvious is simply poor initial electrode placement. However, in previous acute studies in our laboratory where similar microwire arrays were implanted in the lumbar spinal cord using identical placement techniques, the subsequent histology typically revealed a satisfactory level of placement accuracy. The smaller size of the sacral cord, particularly the S2 segment, increases the proportional error, as the targets tend to be smaller than most hindlimb motoneuron pools in the lumbar cord and there is less tissue for the electrode to be mechanically secured in. To achieve stable positioning of the electrode, the electrode lead wire should lay on the surface of the dura mater. For this to occur the distance between the electrode tip and the right-angled bend in the microwire must be accurate to a few hundred microns. This distance could not be accurately predicted for these implants as there can be substantial inter-animal variability in the spinal cord dimensions, rostro-caudal location of the SPN and subdural thickness. To account for this, the electrodes were made longer than necessary to reach their targets and they were glued to the dura mater at the optimal depth during implantation. Pressure on the electrodes from natural movements of the animal as well as growth of connective tissue around the laminectomy site may have caused the electrodes to move which could account for the generally ventral electrode tip positions in Figure 3.2. In some animals, ISMS-evoked responses varied with posture, indicating that different postures caused positional changes in the electrode tips. Finally, there was some evidence that the electrodes may have undergone positional shifts in the spinal cord over the weeks and months after implantation. Across all tested animals, 72% of the electrodes that elicited an increase in bladder pressure greater than 5 mmHg during the first recording session elicited no response during the final session (11.5 ± 11.3 weeks later). In some cases this was due to electrode breakage, but in the majority, a likely explanation was electrode tip migration. During the post-mortem spinal cord dissections it was noted that instead of most electrodes being oriented perpendicular to the dural surface, they penetrated the spinal cord at an angle, sometimes as large as 45 degrees. It is most unlikely that the wires had been implanted at this angle, because considerable care was taken during implantation to insert the microwires perpendicularly. This insertion is difficult to perform perfectly, but a high-powered surgical microscope afforded an excellent view of the microwires and the dural surface. This suggests that the microwires gradually deviated mediolaterally in the spinal cord in the weeks and months post-implantation. If our electrodes had been mounted in a solid base to form a hairbrush-like structure, such as the arrays used by McCreery et al. (2004), it is possible that they would not have deviated as much, either during implantation, or subsequently. On the other hand, hairbrush-like arrays comprised of large numbers of electrodes require relatively powerful, pulsatile insertion. To avoid electrode and tissue damage, the size of such arrays is limited, which in turn reduces the chances of reaching all the desired targets.

It is interesting that in the recent study of Pikov et al. (2007) using this type of array, the electrode positions in the sacral spinal cord did not appear to correspond any better to the targeted nuclei than those in our study. Despite the difficulties encountered in our experiments, several useful points regarding the feasibility of the ISMS technique can be made.

3.4.2 Experimental successes

Bladder contractions were achieved in all implanted animals although there was a large range of maximal bladder pressure that could be elicited by stimulation through a single electrode. The electrodes which were confirmed to be within or in close proximity to the SPN elicited the largest increases in bladder pressure (> 30 mmHg). A number of other electrode positions elicited increases in bladder pressure > 20 mmHg, including the S2 ventral horn and ventrolateral white matter. This is consistent with previous work in which bladder contractions were observed by stimulation in numerous areas other than the SPN (Pikov et al., 2007; Tai et al., 2004). It is also consistent with the known dendritic arborization and spinal axonal pathways of SPN neurons (Nadelhaft et al., 1980) and the pathways of primary bladder afferents within the spinal cord (Morgan et al., 1981). In the instances where several individual electrodes elicited bladder contractions, interleaved stimulation led to increased bladder contractions presumably through greater recruitment of the SPN.

Anesthetic did not seem to affect the maximum bladder contractions elicited by ISMS. This suggests that intra-operative stimulation is an effective way to test the location of an electrode. Given the generally suppressive effects of isoflurane, this result was surprising. In addition, it does not explain why we were unable to elicit bladder contractions intra-operatively in two animals.

A number of electrodes elicited reductions in intraurethral pressure indicating relaxation of the EUS. This relaxation was presumably mediated by interneurons in the DGC. These interneurons are GABAergic, receive direct projections from the pontine micturition center and are believed to project to Onuf's nucleus (Blok et al., 1997; Sie et al., 2001). Since Onuf's nucleus does not receive direct inhibitory projections from supraspinal centers, these inhibitory interneurons may mediate voluntary relaxation of the sphincter (Blok, 2002). Reductions in intraurethral pressure were observed in three animals. Two possibilities exist that could account for the observed relaxation: namely, a reduction in excitatory drive to the Onuf's nucleus or an increase in inhibitory drive to this nucleus. Since micturition is coordinated in the pontine micturition center it is possible that reductions in intraurethral pressure in spinally-intact animals were caused by supraspinally-mediated reductions in EUS drive. However, reductions in intraurethral pressure were also observed after chronic spinal cord transection (see Figure 3.8) suggesting a spinal origin for this result. The pontine micturition center, which can elicit coordinated bladder contractions and reductions in EUS activity when stimulated (Holstege et al., 1986), is known to have direct projections to the SPN but not to Onuf's nucleus (Holstege et al., 1986). Rather, the pontine micturition center has direct connections to GABAergic and glycinergic interneurons in the DGC (Blok et al., 1997; Sie et al., 2001) which in turn form a major source of spinal input to sphincter motoneurons in Onuf's nucleus (Nadelhaft and Vera, 1996). In addition, sphincter motoneurons do not receive other common inhibitory inputs such as recurrent inhibition (Mackel, 1979), crossed inhibition (Jankowska et al., 1978) or cutaneous inhibitory input (Fedirchuk et al., 1992). Reductions in intraurethral pressure can therefore be attributed to stimulation of inhibitory interneurons in the DGC or their axons as they pass to Onuf's nucleus.

ISMS through the same electrodes using the same stimulation parameters elicited similar increases in bladder pressure before and after spinalization in the animal that received a complete transection at T10. This suggests that although numerous plastic changes affecting micturition reflexes occur in the spinal cord after SCI (de Groat and Yoshimura, 2006), these do not affect the ability of ISMS to activate neurons in the SPN that elicit bladder contractions.

3.4.3 Experimental failures

Despite increases in bladder pressure in every animal and reductions in intraurethral pressure in every animal tested for this response, there was a surprising lack of micturition. Recently, Pikov et al. (2007) demonstrated incomplete, but substantial, micturition before and after complete SCI in many of their lightly anesthetized animals using a similar ISMS approach. In our study, the few cases where complete or near complete voiding occurred were from a single spinally intact, awake cat and we suspect that in these instances, the voiding was a voluntary response to the sensations caused by ISMS. This voiding was observed in the animal that subsequently underwent a spinal transection, after which complete or near-complete voiding did not occur. There are several possible explanations for the infrequency of micturition that occurred in our study. The first explanation could simply be that in any one experiment, there were not enough electrodes in the targeted locations (SPN and DGC) to achieve a functional result. A second explanation could be that despite coordinated increases in bladder pressure and decreases in intraurethral pressure such as that shown in Figure 3.6, the bladder pressure was not high enough to overcome static urethral resistance. In anesthetized cats, the urethral pressure profiles suggest static pressures in the range of 30 mmHg in the penile urethra (Lane, 1996; Wang et al., 1999). Similar pressures in the penile urethra during urethral pressure profilometry have also been observed in cats with bilaterally transected pudendal nerves (unpublished observations). This suggests that a minimum bladder pressure increase of approximately 30 mmHg must be achieved to overcome static urethral resistance. Intravesical pressures during voluntary micturition in an awake cat observed during the course of these experiments reached 40 – 57 mmHg, providing an estimate of the bladder pressures required to achieve complete voiding. Since nearly all of our trials in spinally-intact animals were done in the absence of anesthesia, it is possible that the animals were actively opposing attempts to inhibit EUS contractions. Because EUS inhibition is mediated by spinal interneurons, spinally intact animals can directly activate EUS motoneurons via direct connections from the pontine storage center (Holstege et al., 1986). Another simple possibility that could explain the lack of micturition was that ISMS induced concomitant sphincter contractions.

3.4.4 Sensorimotor responses

One motive for performing the majority of the trials without anesthesia was to investigate reflexly- and supraspinally-mediated reactions to ISMS. We found that the majority of the electrodes placed in these experiments elicited unwanted sensory and motor responses at some stimulation amplitude. Movements of the distal leg and tail were frequently observed as well as occasional whole limb flexor responses at higher stimulation amplitudes. However, given that many of the final electrode positions were unknown, and that many of the remaining electrodes were not in their intended targets, it is difficult to determine whether or not sensorimotor responses would be ultimately be problematic. Motor responses have been observed in clinical applications including Nashold's intraspinal stimulation experiments (Nashold et al., 1981) and Brindley's sacral anterior root stimulation implants (Brindley and Rushton, 1990), yet this did not prevent patients from using the devices. We did however notice that a number of electrodes implanted in our study elicited brief vocalizations or orienting responses at low stimulation amplitudes. Overall, 20% of the implanted electrodes elicited these responses. Because the animals never moved away from the test area or changed their resting posture, we concluded that whatever sensations were involved, they did not amount to anything more than mild discomfort. It is difficult to assess whether vocalizations were primarily in response to nociceptive sensations as might be expected if nociceptive transmission neurons in the gray matter were stimulated or the result of unfamiliar innocuous sensations. Stimulation within the dorsal horn and some ascending tracts including the dorsal columns (Palecek, 2004) could also elicit nociceptive sensations.

3.4.5 Lumbar and sacral differences for ISMS

Several differences exist when considering ISMS implants in the lumbar cord for locomotor activation (Guevremont et al., 2006; Mushahwar et al., 2000, 2002) and those intended for implantation in the sacral cord for bladder function. One significant technical difference is simply the size of the relevant part of the spinal cord in the two cases. Since the spinal column is very mobile and the electrode array lead wires "float" on the dura mater, small movements are transmitted to the penetrating portion of the electrode, and proportionally this is likely to be larger in the sacral spinal cord. In addition, electrodes targeting the sacral cord must generally pass through dorsal rootlets which cover this region of the cord. Movement of these rootlets could cause dislodgement of the wires over time. The lumbar cord on the other hand is not generally covered with dorsal rootlets. During implantation, the microwires were rarely aligned perpendicularly to the dural surface prior to insertion, but rather they had to be rotated manually by up to 90° before they were suitably oriented for insertion. The resulting torque, though small, may have been enough to cause gradual migration of the implanted portions of the microwires through the very soft spinal tissue. Small forces developing in the loom leading from the fixation points at the L5 spinous process during activities of daily life may have also contributed to this effect.

Physiological differences also exist between the lumbar targets that activate elements of the locomotor system and the sacral targets for bladder ISMS implants. Locomotor ISMS implants have targeted motoneuron pools whereas ISMS implants for bladder control have targeted small preganglionic neurons to elicit bladder contractions and inhibitory interneurons in the DGC to elicit sphincter relaxation. In some cases a single ISMS electrode has been shown to excite whole populations of motoneurons, eliciting strong single-joint movements or whole-limb synergies (Mushahwar et al., 2000), or in other cases co-contractions of numerous functionally unrelated or antagonistic muscles (Moritz et al., 2007). One potential mechanism for this spread of activation from a single stimulation site to many motoneurons is antidromic activation of muscle spindle primary sensory afferents at or below the threshold for direct activation of motoneurons themselves (Gaunt et al., 2006). ISMS activation of these afferents, which preferentially project to homonymous motoneurons (Mendell and Henneman, 1971), produced sizeable reflex EMG responses in individual muscles. Whether such indirect mechanisms of ISMS activation of target neuron pools exist in the sacral cord for recruitment of SPN neurons is unknown. In this regard it should be noted that the axons of Ia afferents and alpha motoneurons have large diameters (12-20 μm) (Eccles and Sherrington, 1930) and therefore low thresholds to electrical stimulation (Gaunt et al., 2006; Jankowska et al., 1975; McIntyre and Grill, 2000; McIntyre et al., 2004), whereas axons associated with the SPN have a mean diameter of only 1.3 μm, and therefore much higher thresholds (Morgan, 2001).

3.4.6 Future considerations for sacral ISMS for bladder control.

In addition to the challenges for an ISMS-based bladder control neuroprosthesis discussed above, several additional issues must be addressed before ISMS can be considered as a viable approach. It remains to be shown conclusively that stimulation in the DGC can inhibit hyperreflexive EUS contractions in chronic spinal cord injury where detrusor-sphincter dyssynergia is present. Figure 3.8 does demonstrate that reductions in intraurethral pressure can be elicited by ISMS after spinal cord transaction. Pikov et al. (2007) reported improved voiding after SCI with their spinal implant when tested under light propofol anesthesia. However, they also reported that their animals recovered automatic voiding, so the effect of DGC stimulation in cases where the EUS is highly spastic and automatic voiding does not return is unknown. Another common symptom of urinary tract dysfunction after SCI is a hyperreflexive bladder. This neurogenic detrusor overactivity must be suppressed to promote a high-volume, low-pressure bladder. Pikov et al. (2007) reported that ISMS inhibited hyperreflexive bladder contractions in some cases, but there is little additional data on this issue.

In our view, major technological innovations are required to improve ISMS to the point where it could be considered seriously as a basis for a bladder neuroprosthesis in humans. While many of the desired responses, including bladder contractions and urethral inhibition were observed in this study and others (Grill et al., 1999; Pikov et al., 2007; Tai et al., 2004), for clinical purposes, these responses must be obtained in most if not all implants. To achieve this, the primary requirement is reliable targeting and long-term stimulation of specific populations of neurons such as the SPN which is arranged as a thin column up to 10 mm long but as small as 0.2 mm in diameter (Nadelhaft et al., 1980) and surrounded by neurons that ideally should not be stimulated. One suggested approach is to implant high-density electrode arrays, each electrode having multiple contacts along the length of its penetrating shaft (Mushahwar and Horch, 1997). Each shaft should either be independently inserted or be mounted in a suitably flexible substrate that allows groups of electrodes to be implanted together, without damaging the spinal cord on entry and allowing each electrode subsequently to move with its part of the spinal cord so as to avoid migration and damaging tissue with repeated relative movements. Twelve or more shafts (three shafts at each of four rostrocaudal locations in S1 and S2 to target the DGC and the SPN bilaterally) with 4-6 contacts spaced 300 μm apart (to ensure electrode placement within the desired nucleus) would likely be required to guarantee accurate targeting to restore bladder control after spinal cord injury. With this number of electrodes, wiring becomes a substantial problem and wirelessly powered and controlled multi-channel stimulators would probably be required. Unfortunately the insertion of large arrays of suitable electrodes remains problematic. Histological evaluation of the spinal cord, implanted with individual microwires for the purposes of stimulation, has shown that healthy neurons remain in close proximity to the stimulation tips (McCreery et al., 2004; Prochazka et al., 2001). On the other hand, high-density electrode arrays composed of multiple, closely spaced electrode shafts such as the Utah array are generally inserted impulsively, and in the dorsal root ganglion cause substantial tissue damage around each shaft. Shafts become encapsulated to the point that the amount of viable neural tissue available is reduced (Todd, Everaert, Prochazka, Weber and Stein, personal observations). Other histological evidence for the biological response to chronically implant microelectrode arrays comes from the cerebral cortex, and here, reactive responses to the implants can be a major reason for the long term failure of these devices from a recording perspective (Polikov et al., 2005). Fixation of the substrates to the dural or pial surface is also challenging. Even with the use of tissue glue as a fixation means and plastic film to separate the implant from overlying muscle, connective tissue ingrowth tends to lift the substrate from the surface, gradually moving the electrodes away from their targets. Though some progress has been made to address these various problems (McCreery et al., 2004; Snow et al., 2006a,b), much remains to be done before ISMS can be considered a viable clinical approach.

3.4.7 Conclusions

If ISMS for bladder control is to become clinically useful, clear advantages over other electrical stimulation techniques such as sacral root stimulation must be offered. This is especially true as an ISMS implant would likely be at least as difficult to perform as a sacral anterior root stimulator implant. Methods are being developed to overcome the need for the dorsal rhizotomy associated with sacral anterior root stimulation including anodic blocking of large fibers (Rijkhoff et al., 1998) and high-frequency blocking of the pudendal nerves to stop EUS contractions (Bhadra et al., 2006; Boger et al., 2007; Tai et al., 2005). These methods provide an arguably simpler approach to induce bladder contractions without concomitant EUS contractions than ISMS.

Without technical improvements in ISMS electrode arrays that allow more precise targeting, ISMS will probably not be an effective neuroprosthetics technique for bladder control. However, the physiological substrates for an effective device were confirmed in this study. Large increases in bladder pressure (>50 mmHg in some cases) can be elicited by ISMS in awake animals, and chronic spinalization does not impair the ability of ISMS to elicit these contractions. Reductions in intraurethral pressure, indicative of EUS relaxation, were also elicited by ISMS in the sacral cord in chronically implanted animals. However, we were unable to demonstrate substantial micturition in any animal and the unwanted sensorimotor responses combined with the inability to accurately target the intended regions of the spinal cord are a major concern. Whether the lack of voiding was caused by concomitant contraction of the sphincter, insufficient EUS inhibition or voluntary contraction of the EUS by the cat is unknown. In the near future, ISMS for bladder control seems to be a difficult and perhaps ultimately unreliable solution to the problem. Once further development of suitable microelectrode arrays for chronic ISMS implantation occurs, the problem should be re-examined.

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Chapter 4

Intraspinal microstimulation excites multisegmental sensory afferents at lower stimulus levels than local α-motoneuron responses [*]

> *"The very man who has argued you down, will sometimes be found, years later, to have been influenced by what you said."*
>
> – C.S. Lewis

4.1 Introduction

Over the past 10 years, intraspinal microstimulation (ISMS) has been explored as a means of restoring limb movement and bladder control after spinal cord injury (Barbeau et al., 1999; Carter et al., 1995; McCreery et al., 2004; Mushahwar and Horch, 1997, 1998). However, ISMS is in its infancy as a rehabilitation technique and many details surrounding the mechanisms by which ISMS affects activity in the spinal cord and recruits neurons have not been systematically studied.

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In understanding the mechanism of action of ISMS, it is important to know the extent to which ISMS recruits different populations of axons as well as neuronal cell bodies. If large numbers of sensory axons were recruited at lower stimulus intensities than motoneuronal cell bodies or axons, their synaptic action on interneurons as well as motoneurons could greatly affect the motor outcome. As Nowak and Bullier (1998) pointed out "The question that arises is: When a postsynaptic response is obtained after the stimulation of the gray matter, what are the presynaptic neuronal elements activated? The answer to that question is essential, because it will determine the interpretation of the results obtained."

Intraspinal microstimulation was first used as an experimental tool to measure α-motoneuron (MN) synaptic delay to test whether the reflexive activation of MNs by dorsal root stimulation was monosynaptic (Renshaw, 1940). Renshaw showed that at low stimulus strengths, MNs were activated transsynaptically, and at higher strengths they were activated directly. Along similar lines, Jankowska et al. (1975) found that of all the pyramidal tract cells activated with weak microstimulation of the motor cortex in cats and monkeys, only about one third were activated directly. Gustafsson and Jankowska (1976) did a more detailed study of ISMS in cats in which they concluded that MNs were activated directly when the microelectrode tips were adjacent to the initial segment of the MN axon or adjacent to the soma. However, when the tips were amongst MN dendrites and at more distant locations from the soma, MNs were activated transsynaptically. Additional work from the same group has also demonstrated the very low activation thresholds of axons in the central nervous system (CNS) (Jankowska and Roberts, 1972; Roberts and Smith, 1973).

Intraspinal microstimulation has been used by Bizzi and colleagues to study the organization of spinal neural circuitry controlling limb movement (Bizzi et al., 1991). Intraspinal microstimulation in the gray matter of the lumbosacral spinal cord of decerebrate frogs elicited isometric forces in the hindlimb that converged to discrete points. This led to the hypothesis of "movement primitives" in which the spinal cord was suggested to contain four or five basic neural modules producing elemental synergies that could be combined to produce a wide range of movements (Bizzi et al., 2002, 1991; Giszter et al., 1993). In his critique of the concept of movement primitives, Loeb (1992) suggested that the only known topographic structures in the spinal cord are elongated, columnar entities which could not be selectively activated by single microelectrodes. However, Bizzi et al. always assumed that focal ISMS activated sets of interneurons that projected to and activated MN pools in an organized way. From the work of Renshaw and Jankowska and colleagues, one would have expected that sensory afferents would be activated by ISMS, adding synaptic input to MNs. Yet in two sets of experiments ISMS-evoked movement primitives were reported to be similar before and after chronic deafferentation (Giszter et al., 1993; Tresch and Bizzi, 1999). As well, microiontophoretically applied NMDA, which activates cell bodies directly, often produced synergies similar to those produced by ISMS in the same locations (Saltiel et al., 2001). It was argued from these experiments that convergent force fields elicited by ISMS were the result of activating local neurons directly rather than indirectly through incoming axons (Bizzi et al., 1995). Other studies have suggested a modular organization within the dorsal horn of the spinal cord specifically to process cutaneous afferent nociceptive stimuli to coordinate the classical withdrawal reflex (Levinsson et al., 1999; Schouenborg, 2003; Schouenborg et al., 1992; Schouenborg and Kalliomaki, 1990).

Our study was designed to examine the order of recruitment of sensory afferents and MNs (axons or cell bodies), to reveal the rostrocaudal extent of the antidromically activated sensory responses and to characterize and compare the resultant synaptic and direct activation of limb muscles. We found that ISMS at a single point in the spinal cord gray matter activated afferent terminals along the entire length of the lumbosacral enlargement. This casts a new light not only on the mechanism of action of ISMS as a clinical tool, but also on the interpretation of ISMS studies of the organization of the motor elements of the spinal cord. Preliminary results of the present study have been reported in abstract form (Mushahwar et al., 2003).

4.2 Materials and Methods

This report is based on four acute experiments performed to test two hypotheses. 1) As the amplitude of ISMS pulses is increased, afferent axons are activated at lower amplitudes than MNs. 2) Antidromic potentials in sensory afferents have multisegmental reflex effects. Two spinally intact cats (R3 and R4) and two chronically spinalized cats (R1 and R2) were used. Chronic spinalizations were performed to minimize the effects of acute decerebration on the state of the spinal cord and to study ISMS after spinal cord injury, a condition for which ISMS has been proposed as a rehabilitative strategy. The experiments were done with the approval of the University of Alberta Animal Research Ethics Committee.

4.2.1 Surgical procedure

Spinalization

The cats were anesthetized with ketamine (25 mg/kg intramuscular) and intubated using an infant tracheal tube. Pre-operative medication was administered: acepromazine (0.25 mg/kg intramuscular), glycopyrrolate (0.01 mg/kg intramuscular) and buprenorphine (0.01 mg/kg subcutaneous). In addition, the antibiotic cefazolin was administered (10 mg/kg intravenous). The animal's back was shaved, washed with warm soap and water and scrubbed with iodine solution (betadine). Anesthesia was maintained with isoflurane (2-3% in carbogen, flow rate 1500 mL/min). A slow intravenous drip of sterile Ringers solution was administered to maintain fluid balance.

The skin was incised over the T10-L1 spinous processes and a laminectomy was performed at the T10/T11 vertebral junction. The dorsal aspect of the dura mater was incised transversely and a solution of 2% lidocaine (0.2 mL) was dripped on the surface of the cord. Two minutes later, lidocaine was injected into the spinal cord at progressively more ventral levels. Fine scissors were used to transect the cord. The transection was carefully verified visually with a surgical microscope, and a hemostatic mesh, Surgicel (Ethicon Inc., Somerville, NJ), was placed in the gap created by the sectioned spinal cord. The dura mater was sutured shut and the incision was closed in layers. At extubation, the cat was given ketoprofen (2.0 mg/kg subcutaneous). Analgesia was maintained as necessary with ketoprofen (2.0 mg/kg subcutaneous) or buprenorphine (0.01 mg/kg subcutaneous). Cefazolin was administered for 4 days after surgery, followed by amoxicillin (50 mg tablets, 2/day) for 6 additional days. During post-operative recovery the cats were kept warm in heated cages provided with blankets. The animals were allowed to recover for 6-8 weeks before the terminal experiment.

Terminal Experiment

In each experiment the cat was initially anesthetized with the gaseous anesthetic isoflurane (2-3% in carbogen, flow rate 2 L/min). A tracheotomy was performed and a tracheal tube was inserted to allow control of ventilation with a closed-loop anesthetic machine. The carotid artery was ligated on one side and catheterized on the other to allow monitoring of blood pressure. The jugular vein was catheterized to allow drug administration.

A laminectomy was performed to expose the L6-S1 region of the spinal cord and associated dorsal roots after which the cat was fixed in a stereotaxic frame. An array of 4-6 microwires was inserted through the dura mater into the L5-L7 spinal cord (R1 – 4 electrodes in L6-L7, R2 – 6 electrodes in L6, R3 – 6 electrodes in L5, R4 – 6 electrodes in L6). The microwires were made from 25 or 30 μm diameter stainless steel insulated with polyimide with 60–100 μm of wire bared at the tip and cut with a sharp scalpel blade to have an acutely angled bevel. Once the wires were inserted into the spinal cord, the array was fixed to the dura mater and the L3 spinous process with droplets of Loctite 420 cyanoacrylate glue. The depths of insertion of the microwires from the cord dorsum ranged from 1.75 to 3.5 mm, targeting the dorsal horn (lamina IV, V, VI), intermediate gray matter (lamina VII) and the ventral horn (lamina IX). The locations of the electrode tips established in post-mortem dissections of the spinal cord are shown in Figure 4.1. Electrode positions were identified by serial sectioning of the formalin-fixed spinal cord. Once an electrode was visualized, the size and shape of the white and gray matter were recorded as well as the position of the electrode tip within the gray matter. In cat R4, the ISMS electrodes were dislodged from the spinal cord at the termination of the experiment. However, based on this and previous work, we are confident that these electrodes were within the gray matter and evenly distributed from the dorsal to ventral horn.

Bipolar EMG electrodes (Cooner AS631) were implanted in 5 to 8 of the following hindlimb muscles ipsilateral to the ISMS electrodes: lateral gastrocnemius (LG), medial gastrocnemius (MG), tibialis anterior (TA), biceps femoris posterior (BFp), biceps femoris anterior (BFa), vastus lateralis (VL), sartorius anterior (Sart), and semimembranosus anterior (SMa). The inter-electrode spacing was approximately 2 cm. The hip, knee and ankle joints of the leg ipsilateral to the ISMS electrodes

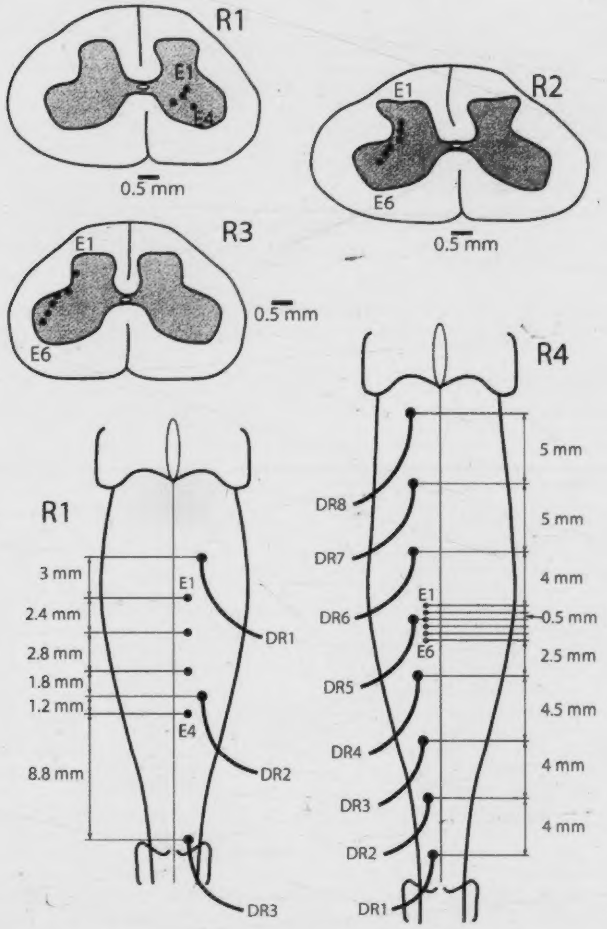

Figure 4.1: *ISMS electrode locations and DR recording sites*

Electrode locations in three of the four cats. In addition to the dorso-ventral spread shown in the top half of the figure, electrodes were distributed rostrocaudally with the most rostral electrode also being the most dorsal and the most caudal electrode also being the most ventral. The rostrocaudal interelectrode spacing was 0.5 mm in cat R3 and R4, 1 mm in cat R2 and varied in cat R1 (see diagram). In the cat R4, the ISMS electrodes were dislodged from the spinal cord at the conclusion of the experiment and therefore their exact locations could not be verified. The rostrocaudal location of the electrodes in relation to the recorded dorsal rootlets is shown for cats R1 and R4 in the lower half of the figure. Similar diagrams for cats R2 and R3 and shown in Figure 4.5 and Figure 4.6 respectively.

were fixed by clamps to the base of the stereotaxic frame to minimize movement during ISMS trials. A heating blanket under the abdomen was used to maintain body temperature. The skin at the laminectomy site was attached to the frame with elastic bands to form a pool that was filled with clear paraffin oil to cover the exposed spinal cord. A heating lamp was used to maintain the temperature of the paraffin oil close to body temperature.

A mid-collicular decerebration was performed and isoflurane anesthesia was discontinued. Decerebration abolishes consciousness but spares basic motor functions mediated by the brainstem and spinal cord. A period of 1-2 hours was allowed for the effects of the anesthetic on the spinal cord to wear off. After hindlimb tone and reflexes had re-appeared, ISMS pulses were applied through each of the implanted microwires. Antidromic responses were recorded in cut dorsal root filaments with bipolar platinum hook electrodes. Filaments were selected according to the distance of their cord entry points rostral and caudal to the stimulation sites. Motoneuron activation was recorded indirectly in the form of EMG responses in the hindlimb muscles implanted with EMG electrodes. Figure 4.2 provides a schematic of the experimental setup. Stimulus-response properties of the dorsal root and EMG responses were characterized for a range of ISMS intensities. In one cat, data were obtained under isoflurane anesthesia before decerebration as well as after decerebration in the absence of anesthesia.

4.2.2 Data recording and analysis

Recording, sampling and stimulation

Electroneurograms (ENGs) from the cut dorsal root filaments were amplified with an Iso-DAM8A differential instrumentation amplifier (World Precision Instruments, Sarasota, FL) at a gain of 10,000 and band-pass filtered from 300-3000 Hz with a 20 db/decade roll-off. Hindlimb EMGs were amplified with Neurolog NL824 preamplifiers and NL820A isolator units (Digitimer Ltd., Welwyn Garden City UK) at a gain of 1000 and band-pass filtered from 100-2000 Hz with a 20 db/decade roll-off using custom-built filters. All signals were displayed on Tektronix analog oscilloscopes, digitally sampled at 20,000 samples per second and stored with the use of a CED 1401plus Laboratory Inter-

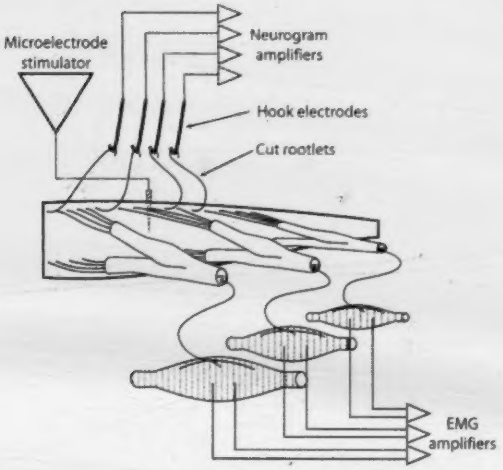

Figure 4.2: *Schematic of the experimental setup*

The locations of ISMS electrodes, dorsal roots, dorsal root recording hook electrodes and implanted EMG electrodes are shown. For clarity, only one of the ISMS electrodes is shown.

face (Cambridge Electronic Design Ltd., Cambridge, UK). Sampling was peri-triggered with 5-20 ms of pre-stimulus data and 10-20 ms of post-stimulus data. Neurolog modules NL304 (period generator), NL403 (delay-width), NL510 (pulse buffer) and NL800 (stimulus isolator) were used to deliver 200 μs long, constant current monophasic pulses through the ISMS electrodes at 1 pulse per second. Each trial consisted of 50 to 250 individual records and this was repeated for each stimulation amplitude. Stimulation amplitudes ranged from 20 – 300 μA depending on the animal and location of the microwire within the spinal cord. The stimulus amplitude was slowly increased from zero until any response was observed visually on oscilloscopes displaying dorsal root ENG and hindlimb muscle EMG waveforms. Sampling was commenced at this amplitude and proceeded at increasing values until both the pattern of ENG and EMG activity did not change appreciably with increased stimulation amplitude or when the stimulation current reached 300 μA.

Automatic threshold detection

The primary aim of this study was to compare the stimulus amplitudes first eliciting ENGs and EMGs. The progression of EMG onset response latencies was also of interest. To eliminate qualitative judgment of EMG onset, the presence of activity and its corresponding onset latency was automated using custom software in Matlab v6.5 (The Mathworks Inc., Natick, MA). The data were pre-processed and any DC offset present in the pre-stimulus data was removed from each record individually. Each record was then rectified and analysis was resumed.

The two-sample t-test was used to detect the onset latencies of ENG and EMG signals. The null hypothesis was that the distribution of data from all records of a given signal at a given post-stimulus latency was equal to the distribution of data from all pre-stimulus times (significance at $p<0.001$). Each post-stimulus latency was tested in this way. Consider a data set in which 80 records of responses to the same stimuli were obtained with 20 ms of pre-stimulus data and 20 ms post-stimulus, sampled at 20,000/s. For each latency, the two distributions for t-test analysis would comprise 32,000 pre-stimulus points and 80 post-stimulus points. The first 0.5 ms of post-stimulus ENG signal containing the stimulus artifact was neglected, as was the first 2 ms of EMG signal (the minimal possible response latency). Finally, the criterion for detecting a response onset was that consecutive data points spanning at least 0.5 ms of the ENG signal and 1 ms of the EMG signal reached significance. The difference in window length for ENG and EMG signals was selected to minimize false positive detections. Since the longer window for EMG activity might bias detection towards higher stimulation amplitudes, all collected data were also analyzed with an EMG detection window of 0.5 ms. In this scenario, EMG signals were occasionally detected at lower thresholds, however visual inspection of these data often did not positively identify activity. Additionally, in no case did this change reduce any EMG thresholds to be equal to or less than the ENG thresholds. Because of this, the EMG detection window was left at 1.0 ms.

Figure 4.3 shows an example of the processing and automatic detection of ENG and EMG signals in one data set. The stimulus artifacts in the ENG signals can be clearly seen and in this example an ENG signal was detected in dorsal roots 2-4. Four of the eight recorded EMG channels are also shown. An EMG signal was detected in LG, MG and TA, but not in VL.

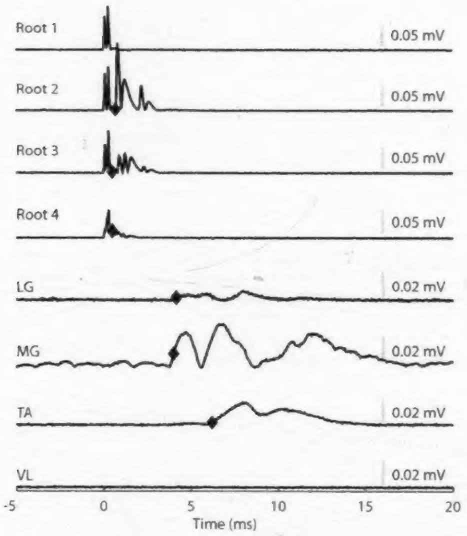

Figure 4.3: *Dorsal root ENG and hindlimb EMG responses to ISMS*

Averaged dorsal root ENGs (upper four traces) and hindlimb EMGs (lower four traces) in response to an ISMS pulse supra-threshold for both sensory and motor responses. The stimulation pulse was delivered at time t = 0 s. The diamonds mark the location of automatically detected activity onsets. The large spikes at the beginning of the ENG signals are stimulus artifacts. No activity was detected in dorsal root 1 or VL in this case. Note the difference in onset latency between EMG and ENG signals.

4.3 Results

4.3.1 ISMS stimulates afferent fibers at lower amplitudes than local motoneuron cell bodies or axons

Figure 4.4 shows an example of a sequence of trials in cat R3 in which ISMS intensity was increased in steps from 35 μA to 150 μA. Each trace is an average of responses to approximately 110-220 stimuli. Six ISMS stimulus intensities are represented. At the lowest intensity, ENG responses are evident in two of the three dorsal root filaments while no EMG responses are seen. As ISMS amplitude was increased, the amplitudes of dorsal root responses increased and EMG responses appeared and grew in amplitude. The data in Figure 4.4 were typical of nearly all trials in that dorsal root responses occurred at lower stimulation intensities than EMG responses.

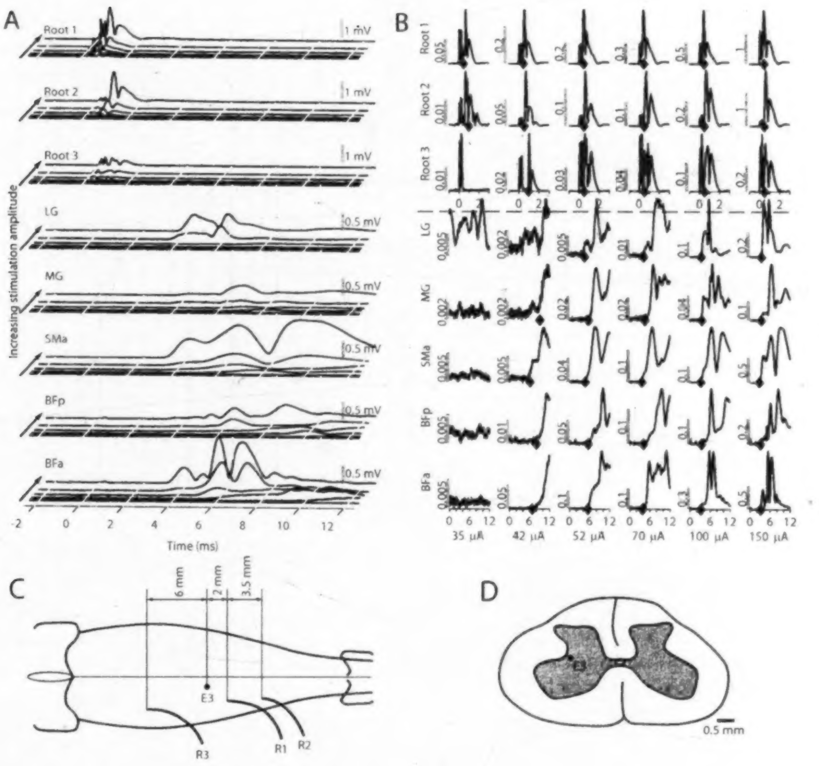

Figure 4.4: *Dorsal root ENG and hindlimb EMG responses to increasing ISMS amplitude*

A sequence of dorsal root ENG and hindlimb EMG responses to increasing stimulation amplitudes through an ISMS electrode in the intermediate region of chronically spinalized animal R2. The stimulation amplitude was increased from 35 μA to 150 μA during the trial. In each case the plotted trace is the average of approximately 110-220 stimuli. Each row is labeled with the dorsal root or muscle that the signal was recorded from. This progression of responses was typical amongst all cats and experimental parameters. (A) The progression of responses plotted on the same vertical scale for the duration of the trial. Stimulation amplitude increases in the direction noted to the left of each panel. The increase in the magnitude of the responses in both the dorsal roots and muscles can be clearly seen. In the muscles, the progression from long latency responses at low stimulation amplitudes to short latency responses at higher stimulation amplitudes can also be seen. (B) The data from part (A) plotted on varying scales so that the details of the responses can be visualized. Each row is labeled with the dorsal root or muscle the data were recorded from and correspond to the rows of part (A). Each column presents data from different stimulation amplitudes. The amplitude scale is plotted in each panel in mV. Note that the time scales for the dorsal root signals and EMG signals are different, but are both in milliseconds. In each case where activity was determined to have occurred, a diamond is plotted at the onset of activity. At the lowest intensity, responses are evident in two of the three dorsal root filaments, in the absence of detectable EMG responses. As ISMS intensity was gradually increased, the amplitudes of dorsal root responses increased and EMG responses appeared and grew in amplitude. (C) Schematic showing the location of the ISMS electrode (E3) and recorded dorsal rootlets. (D) Cross section of the spinal cord showing the location of the ISMS electrode tip (E3).

Figure 4.5 presents the complete data set for chronically spinalized cat R2. The six panels show response thresholds for dorsal root ENGs and hindlimb muscle EMGs for each of the 6 microwires through which ISMS was delivered. Electrode E1 was the most dorsal electrode and E6 was the most ventral electrode. The onset latencies for EMG data are also shown. The response thresholds for the three dorsal roots sampled in this cat are indicated by arrows along the x-axis. Electromyogram responses from each muscle are plotted as response latency versus stimulation amplitude. In this cat, ENG responses were always detected in some dorsal roots before any EMG activity even for the deepest stimulating electrode. The first dorsal root ENG response was detected at a mean stimulus amplitude of 28 ± 2 (SD) μA. One might presume that deeper stimulating electrodes would be closer to MN cell bodies and their axons and thus activate MNs (somatically or axonally) at lower stimulation intensities. However, there was no evidence for this in cat R2, even though this progression of responses was observed in other animals (see below). In fact, even with the deepest electrode, the EMG onset latencies suggest that MNs were first activated indirectly by transsynaptic pathways.

As the stimulation amplitude was increased, it was also generally observed that the EMG response latency decreased in most muscles. Across all animals and all conditions, linear regression of the pooled data indicated that EMG latency did in fact decrease with increasing stimulation amplitude (ANOVA $p < 0.001$). However, less than 10% of the variance of pooled data was accounted for by the regression ($r^2 = 0.075$). At the highest ISMS amplitudes, the measured EMG latencies were 3-4 ms when ISMS was delivered through the more dorsal electrodes and gradually decreased to 2 ms for the most ventral electrode. Even though 4 ms is not an unreasonable time for detection of EMG with direct MN stimulation assuming a conduction velocity of 60 mm/ms, 200 mm of axon and a delay of 0.5 ms at the neuromuscular junction, the decrease in onset latency from dorsal to ventral suggests transsynaptic activation of MNs with the dorsal electrodes. This seems reasonable since no MNs are present in the dorsal horn and estimations of current spread in spinal gray matter from a microelectrode are around 0.5-1 mm at 150 μA (Gustafsson and Jankowska, 1976; Ranck, 1975; Snow et al., 2006). With this amount of current spread, it is unlikely that MNs could be activated directly. More interestingly, at lower ISMS amplitudes, EMG onset latencies were much higher (4-10 ms) indicating that MNs were almost certainly activated transsynaptically. These responses are likely

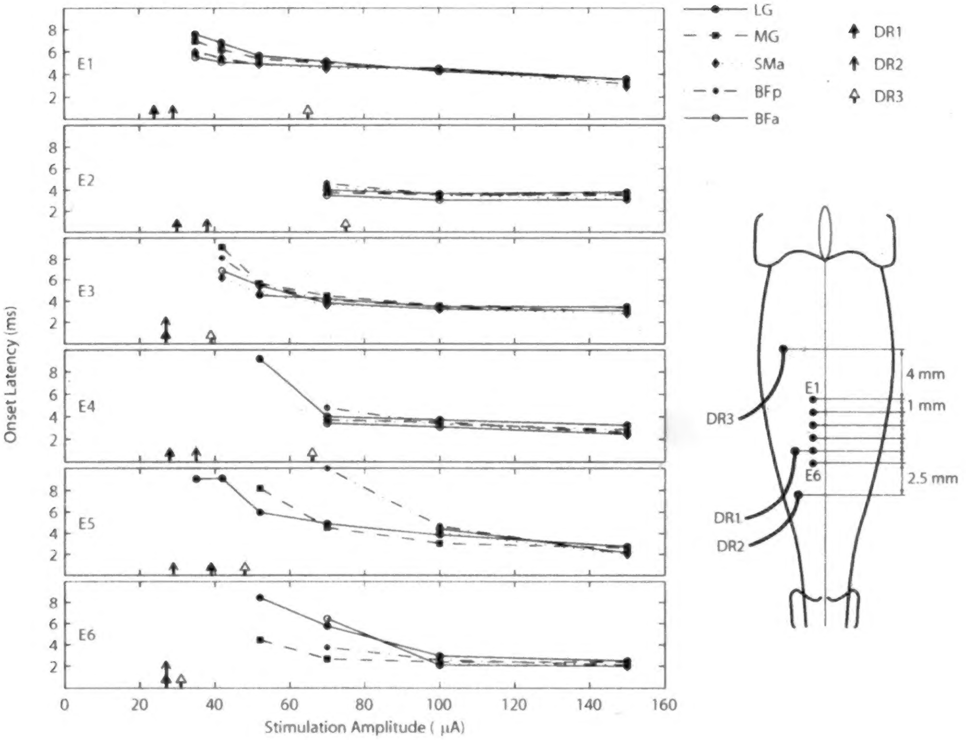

Figure 4.5: *Dorsal root ENG and hindlimb EMG response thresholds in cat R2*

Stimulation thresholds of the dorsal root ENGs and hindlimb EMGs as well as the latencies of EMG responses for each ISMS electrode in chronically spinalized cat R2. Each panel presents the data for one of the six ISMS electrodes (E1-E6). E1 was the most rostral and dorsal electrode while E6 was the most caudal and ventral electrode. Arrows indicate the stimulation amplitude at which each of the dorsal roots first exhibited detectable ENGs. Stacked arrows indicate multiple dorsal roots with the same stimulation threshold. The roots are identified by distinctive arrow styles. The onset latencies for each muscle are also displayed as a function of stimulation amplitude. For example, in panel E5, LG first exhibited detectable activity at a stimulation amplitude of 35 μA and at a latency of 9 ms whereas MG first exhibited detectable activity at a stimulation amplitude of 52 μA and at a latency of 8 ms. The relative location of the stimulation electrodes and the dorsal rootlets recorded from are also shown. Note that in all cases, activity was detected in some dorsal root ENGs at lower stimulation amplitudes than hindlimb EMGs.

mediated by at least some of the sensory afferents whose antidromic responses were recorded in the dorsal roots.

Figure 4.6 presents the complete data set for cat R3, one of the two spinally intact cats, in the same format as Figure 4.5. In this cat, the differences between the thresholds of dorsal root ENGs and hindlimb EMGs ranged more widely than those in R2. For example, electrode E1 elicited the first dorsal root response at 39 μA but the first EMG response did not appear until 90 μA. The deeper electrode, E5, elicited the first dorsal root response at 31 μA and the first EMG response at 33 μA. This decrease in EMG threshold as the electrode tips neared MNs is what would be expected from Gustafsson and Jankowska (1976), unlike the findings in animal R2. When stimulating through electrode E6, the deepest of the ISMS electrodes, the threshold for TA EMG was 31 μA while the minimum dorsal root ENG threshold was 36 μA. In this instance, hindlimb EMG activity was detected at a lower stimulus intensity than dorsal root ENG activity.

Figure 4.7 shows a summary of the results obtained from each cat. Afferents were activated at lower thresholds than MNs, the only exceptions being the deepest electrodes in cats R3 and R4. Even in these cases, the difference in thresholds between the first EMG and first ENG was only 3-5 μA. This implies that in general, ISMS did not activate MNs without also antidromically activating local afferents, whose arborizations extended rostrally and caudally in the spinal cord.

In addition to activating afferent fibers at lower amplitudes than MN cell bodies or axons, ISMS in the spinal gray matter may activate both ascending and descending propriospinal pathways as well as local interneurons. The possible effect of ISMS-activated descending pathways on dorsal rootlet thresholds as well as EMG thresholds and latency at threshold was measured using a Mann-Whitney Rank Sum test to compare the spinally intact group to the chronically spinalized group in which descending contributions are absent. Statistically significant differences were found between the spinal and non-spinal cats in the dorsal rootlet ENG thresholds (intact 63.6 ± 40.2 μA, spinalized 51.4 ± 20.8 μA, $p = 0.046$) hindlimb muscle EMG thresholds (intact 125.2 ± 71.0 μA, spinalized 109.9 ± 70.8 μA, $p = 0.037$), but not in the EMG latency at threshold (intact 5.8 ± 3.4 ms, spinalized 5.3 ± 2.3 ms, $p = 0.86$). A number of non-neurophysiological explanations may account for these differences. In the spinally intact cats, the rostrocaudal extent of dorsal rootlets recorded from was greater and these

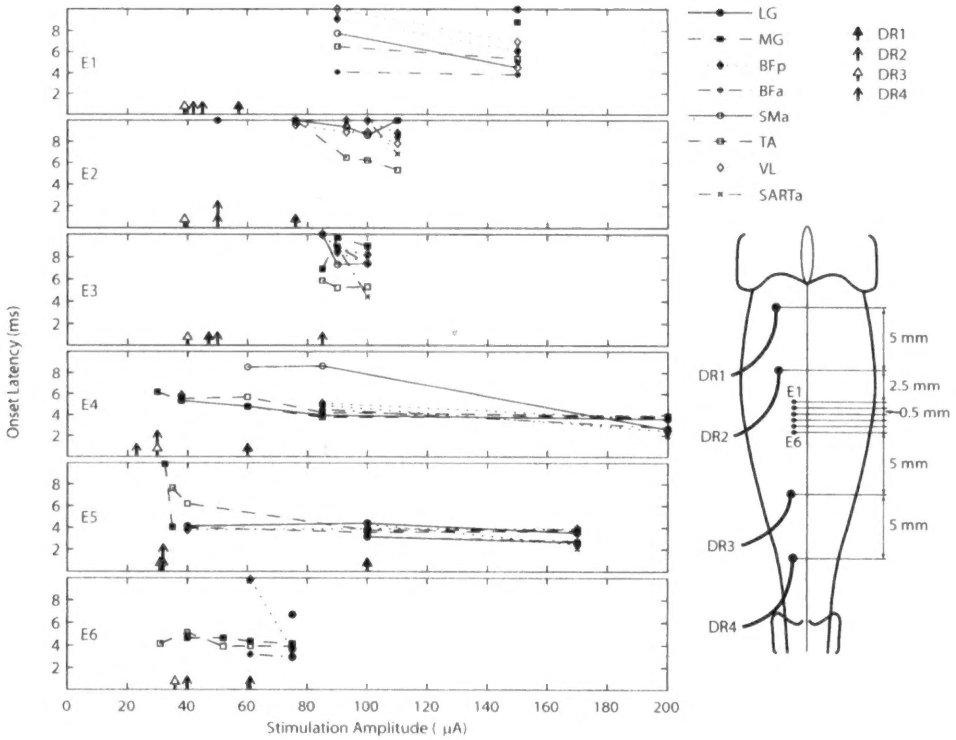

Figure 4.6: *Dorsal root ENG and hindlimb EMG response thresholds in cat R3*

Stimulation thresholds of the dorsal root ENGs and hindlimb EMGs as well as the latencies of EMG responses for each ISMS electrode in spinally intact cat R3. Each panel presents the data for one of the six ISMS electrodes (E1-E6). E1 was the most rostral and dorsal electrode while E6 was the most caudal and ventral electrode. Arrows indicate the stimulation amplitude at which each of the dorsal roots first exhibited detectable ENGs. Stacked arrows indicate multiple dorsal roots with the same stimulation threshold. The roots are identified by distinctive arrow styles. The onset latencies for each muscle are also displayed as a function of stimulation amplitude. For example, in panel E4, MG first exhibited detectable activity at a stimulation amplitude of 30 μA and at a latency of 6 ms whereas SMa first exhibited detectable activity at a stimulation amplitude of 60 μA and at a latency of 8.5 ms. The relative location of the stimulation electrodes and the dorsal rootlets recorded from are also shown. Note that in electrodes E1-E5, activity was detected in dorsal root ENGs at lower stimulation amplitudes than hindlimb EMGs.

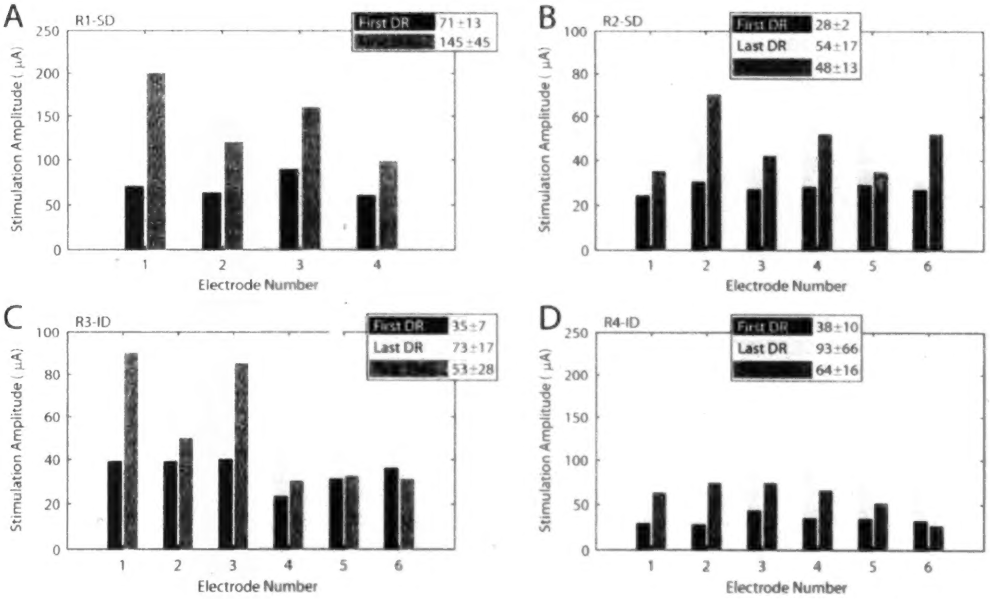

Figure 4.7: *Dorsal root ENG and hindlimb EMG threshold summary*

Differences between stimulation amplitude required to elicit dorsal root ENGs and hindlimb EMGs depending on the stimulating electrode. The "First DR" response was the minimum stimulation amplitude at which compound action potentials were recorded from any of the dorsal roots. The "Last DR" response was the minimum stimulation amplitude at which action potentials were recorded on all dorsal roots. The "First EMG" response was the minimum stimulation amplitude at which EMG could be recorded from any muscle. In the legend of each panel, the mean and standard deviation of each of the three parameters are given as calculated over the range of electrodes. Above each panel, the animal is named and indicated as being spinally intact (I) or chronically spinalized (S) and decerebrate (D). (A) In chronically spinalized cat R1, sensory axons were always antidromically stimulated before MNs were activated. (B) In chronically spinalized cat R2, sensory axons were always antidromically stimulated before MNs were activated, even with the deep electrodes in the ventral horn. (C) In spinally intact cat R3, sensory axons were always antidromically stimulated before MNs were activated with the exception of the deepest electrode (E6) where the first EMG signal had a threshold of 31 μA and the first dorsal root ENG had a threshold of 36 μA. The thresholds for compound dorsal root action potentials and EMG were essentially the same for second deepest electrode (E5): 31 μA for the first ENG and 32.5 μA for the first EMG. (D) In spinally intact cat R4, sensory axons were always antidromically stimulated before MNs were activated with the exception of the deepest electrode (E6).

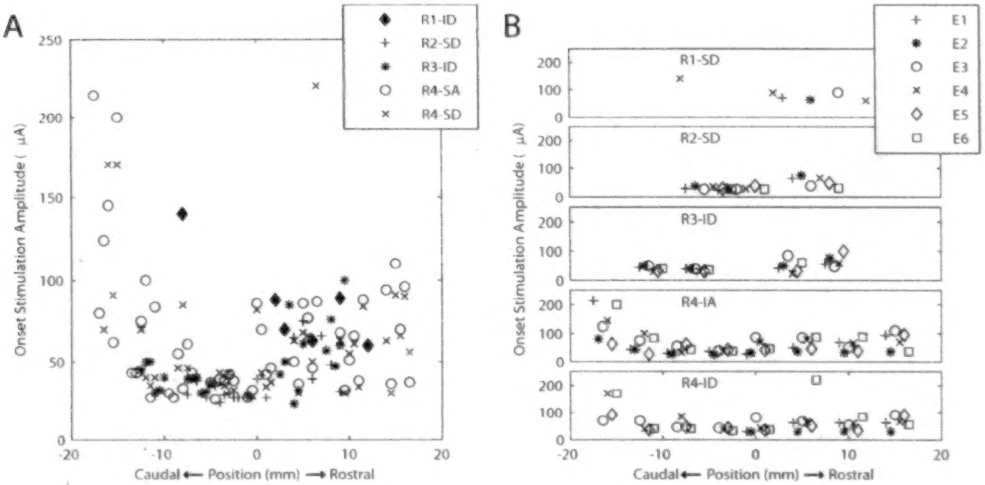

Figure 4.8: *Spread of ISMS through multisegmental afferents*

Antidromic spread of activity through sensory axons in the spinal cord. The relationship between the minimum ISMS amplitude required to elicit a compound action potential in a specific dorsal root and the distance between the ISMS electrode and the dorsal root entry zone is shown. Compound dorsal root action potentials were recorded up to ± 17 mm away from the stimulating electrode. Animals are listed (R1-R4) and indicated as being spinally intact (I) or chronically spinalized (S) and anesthetized (A) or decerebrate (D). (A) Pooled data from all four animals. All dorsal roots and electrodes are shown. (B) The same data as displayed in (A), but showing the rostrocaudal spread of activity from each stimulating electrode for each animal.

distant rootlets generally had higher thresholds. Also, the locations of ISMS electrodes were not consistent between experiments, potentially leading to differences in EMG thresholds not related to the spinal state of the animal.

4.3.2 Spread of focal stimulation in the spinal cord

The rostrocaudal extent to which compound dorsal root action potentials could be recorded was larger than the length of the lumbosacral enlargement. Figure 4.8 shows the response thresholds of dorsal rootlet ENGs as a function of the rostrocaudal distance between the rootlet entry zone into the spinal cord and the ISMS electrode. The data from all four cats are combined in panel A. The most striking feature of this plot is the ± 17 mm extent of dorsal root responses to focal ISMS (farther distances were not explored).

Figure 4.8 shows that as the distance from the stimulation location (shown as zero) increased, the average stimulation amplitude required to elicit a response in the dorsal roots increased. Dorsal rootlets entering the spinal cord at the position of the ISMS electrode and up to 5 mm caudally all exhibited detectable ENGs between 24 μA and 43 μA. Caudally, the stimulation threshold to elicit ENGs increased to 62 - 214 μA for dorsal roots 15 - 17 mm from the stimulating electrode site. Rostrally, the stimulation threshold to elicit ENGs was 30 - 110 μA for dorsal roots 15 - 17 mm from the stimulating electrode with the exception of a single outlying point. For typical ISMS amplitudes (50 μA - 250 μA) used to generate functional movement with microwires of the type we used (Mushahwar et al., 2000), afferent backfiring would cause the focally applied stimulation to excite regions of the spinal cord at least ± 17 mm away from the stimulation site. Note that ± 17 mm refers to the most rostral and caudal dorsal root entry points. Panel B of Figure 4.8 presents the same data as panel A, but the spread elicited by each electrode for each cat can be individually seen. To examine potential differences between the anesthetized and decerebrate case in animal R4, both linear and quadratic regression lines were fitted to each of these data sets. The regression lines were compared using an F test and in neither case ($p = 0.88$ linear, $p = 0.89$ quadratic) was there a significant difference between the anesthetized and decerebrate state. Additionally, no obvious relationship between the stimulating electrode depth and the dorsal rootlet ENG threshold at different rostrocaudal locations was found. The fourth panel of Figure 4.8B (R4-SA) shows that the dorsal rootlet 15-17 mm caudal to the ISMS array exhibited compound action potentials in response to stimulation through electrodes E2 and E5 at amplitudes from 50-100 μA, whereas the same rootlet only exhibited compound action potentials at stimulation amplitudes greater than 200 μA with electrodes E1 and E6. Dorsal rootlets close to the site of ISMS exhibited compound action potentials within a much narrower range of stimulation amplitudes, presumably because of the higher density of collaterals in this region.

One obvious feature of Figure 4.8A is the apparent asymmetry in the range and distribution of ISMS amplitudes which first elicited a dorsal root response. Dorsal rootlets rostral to the ISMS electrode maintained a relatively consistent distribution of thresholds beginning immediately rostral to the ISMS electrode. Caudal to the ISMS electrode, the dorsal rootlet thresholds were consistently low for animals R2 and R3, but for animals R1 and R4 began to increase rapidly to over 200 μA beginning 7 mm caudal to the electrodes. This effect was primarily observed in animal R4 where the examined rootlets were up to 17 mm caudal to the ISMS electrodes.

4.4 Discussion

The main finding of this study was that sensory afferents were nearly always activated at lower ISMS thresholds than MNs (cell bodies or axons), whether the electrode tips were located in the dorsal horn, intermediate gray matter or ventral horn. This is consistent with previous work showing that at low stimulation levels, ISMS activates MNs transsynaptically except when applied in close proximity to the initial segments of MN axons (Gustafsson and Jankowska, 1976). What is new in this study is the demonstration of a very extensive spread of activity resulting from the antidromic activation of afferent axons with focal ISMS, especially with the ISMS electrodes in the ventral horn. Responses were detected in dorsal root filaments with entry zones 17 mm rostral and 17 mm caudal to a single microwire delivering ISMS pulses. This result suggests that when ISMS was applied at a given point within the gray matter of the spinal cord, antidromic action potentials were elicited in terminal branches of sensory afferents close to the electrode tip. These action potentials propagated back to their parent axons and via dorsal columns to dorsal root filaments, where our recording electrodes were located. In addition, the fact that many muscles showed responses at polysynaptic latencies, suggests that the action potentials also propagated rostrally and caudally along the dorsal columns and re-entered the gray matter at other collaterals of the afferents. Rootlets farther from the ISMS electrodes were not examined. This amount of stimulus spread is greater than the entire length of the lumbosacral enlargement (28.8 mm ± 2.4 mm) (Vanderhorst and Holstege, 1997).

Horseradish peroxidase tracing studies by Brown (1981) have shown that sensory afferent collaterals can be found entering the spinal cord gray matter up to 10 mm rostrally and 5 mm caudally from their entry zones. The existence of afferent collaterals projecting from the L2-4 spinal segments to at least S1 has also been demonstrated electrophysiologically (Wall and Werman, 1976) and by axonal degeneration (Imai and Kusama, 1969). Furthermore, branches of spindle afferents are known to synaptically excite most if not all homonymous MNs (Mendell and Henneman, 1971). Also, dorsal horn interneurons respond to input from afferents with entry points up to 3 segments away (Mendell et al., 1978). These facts indicate that what is presumed to be focal stimulation of the spinal cord in fact probably excites both motoneurons and interneurons over large distances in all directions via antidromic activity in afferent fibers. In addition to the activation of sensory afferents, ISMS presumably recruits propriospinal axons in the vicinity of the electrode tips which may be important in contributing to the net motor output. Long ranging propriospinal neurons with terminations in the lumbosacral enlargement are known to exist (Jankowska et al., 1974), but are unlikely to be the cause of the widespread activation of dorsal rootlets in this study.

These results were neither obvious to us in our own previous ISMS studies, nor was the widespread activation of the spinal cord by ISMS recognized at the outset as a possible factor in the work that led to the hypothesis of movement primitives (Bizzi et al., 1991). Movement primitives have been observed after chronic deafferentation (Giszter et al., 1993; Tresch and Bizzi, 1999), but the primitives themselves were somewhat changed from those observed with intact afferent input. ISMS, particularly in the dorsal horn and intermediate region may also activate cutaneous nociceptive afferents that activate columnar somatotopically organized modules that coordinate the withdrawal reflex (Schouenborg, 2003). The modules have been demonstrated in the rat (Schouenborg et al., 1992; Schouenborg and Kalliomaki, 1990; Schouenborg and Weng, 1994) and cat (Levinsson et al., 1999) and are present after spinalization, although the specificity of the receptive field mapping degrades over time (Schouenborg et al., 1992) The relationship between these reflex modules and the theory of movement primitives is unclear. These observations, in combination with our results of widespread activity caused by antidromically activated afferents suggests that the interpretation of the results of ISMS in the presence of intact afferent fibers is challenging. When afferent fibers are intact, the transsynaptic activation of MNs elicited by antidromic activation of sensory afferents in response to ISMS is bound to affect movements in a way that may not occur in the normally functioning nervous system. This could also partly explain the results of Mushahwar et al. (2004) in which ISMS in the intermediate gray matter elicited responses that were significantly modified by changes in descending input to the spinal cord caused by decerebration and acute spinalization.

The experimental demonstration that focal stimulation excites a whole population of axons at lower intensities than a co-localized population of neuronal cell bodies has broader implications. Currently, the mechanism by which deep brain stimulation operates is unknown (Dostrovsky and Lozano, 2002). However, theoretical work (McIntyre and Grill, 2000; McIntyre et al., 2004), experiments involving intracortical stimulation (Jankowska et al., 1975) and in vitro experimental data (Nowak and Bullier, 1998) all suggest that electrical stimulation in the CNS, notably in the basal ganglia, excites axons at lower intensities than local neuronal cell bodies. To demonstrate this in vivo and to examine the relative recruitment of axons versus neuronal cell bodies, the neuroanatomy has to be such that there is separate electrophysiological access to the two populations. With current technology, this is probably only possible in the spinal cord, where the activity of afferent axons is selectively accessible in the dorsal roots, and the activity of MNs is selectively accessible either via their axons in the ventral roots, or via the muscles to which they project. Our study strongly supports both the theoretical and experimental work suggesting that in the CNS, large populations of axons around a microelectrode are activated at lower thresholds than nearby cell bodies. Additionally, these results suggest that ISMS antidromically activates not only sensory axons but could also activate interneurons and *en passant* axons of other neurons that could in turn excite or inhibit neurons elsewhere in the network.

In addition to theoretical (McIntyre and Grill, 2000) and experimental (Gustafsson and Jankowska, 1976) evidence suggesting that axons are activated before cell bodies, one factor that might bias excitation towards the sensory axons in ISMS is the small number of MN cell bodies and axons close to a microwire tip compared to the number of afferents with branches in the same volume of gray matter. Given that a single MN receives synaptic input from many afferents, the density of terminal branches of afferent axons in any focal volume of ventral horn is sure to be much higher than the density of MNs. This might explain why MN cell bodies and their axons were usually excited at higher thresholds than sensory axons. It is also consistent with the observation that the difference between thresholds for dorsal root potentials and EMG was generally smaller when electrodes were positioned in the ventral horn. In two cases (see Figure 4.7), the threshold for EMG detection was essentially equal to or even slightly below that for dorsal root potentials. At low stimulation amplitudes in the ventral horn, it is probable that MN axons rather than MN cell bodies themselves were activated by electrical stimulation. In fact ISMS-elicited action potentials leading to short-latency EMG responses may have originated in the MN axons in all cases (Gustafsson and Jankowska, 1976; McIntyre et al., 2004). Other factors that may bias excitation preferentially towards sensory axons rather than motor axons include the observed differences in the time constants of sensory and motor axons as well as primary afferent depolarization (PAD) caused by presynaptic inhibition. Erlanger and Blair (1938) first reported preferential activation of motor or sensory axons in the trunks of bullfrog spinal roots depending on the stimulation pulse width. At short pulse widths, motor axons were preferentially activated, while at longer pulse widths, sensory axons were preferentially activated. This phenomenon exists in human peripheral nerve as well, and Panizza et al. (1992) have demonstrated that the pulse widths at which the motor and sensory thresholds are equivalent in the ulnar and median nerves are 200 μs and 300 μs respectively. The reason for this difference has been postulated to be a higher percentage of persistent Na^+ channels at the nodes of Ranvier of sensory axons (Bostock and Rothwell, 1997). Whether these effects are present in the spinal cord is unknown, however, the pulse width used in our experiments (200 μs) is in the range where differences between sensory and motor thresholds was minimal in peripheral nerves. PAD could also have an effect on the observed differences between sensory and motor activation thresholds (Rudomin and Schmidt, 1999). PAD would bias activation thresholds towards lower sensory axon thresholds while also increasing the threshold for reflexly evoked motor activity.

In this study, single shocks were used, rather than trains of pulses as would typically be used in a neuroprosthesis application. Trains of pulses would have likely lowered the threshold for axonal activation (Gustafsson and Jankowska, 1976), and would therefore have recruited both afferent axons, efferent axons and any transsynaptic efferent responses at lower stimulation amplitudes. Whether there might have been a differential effect on afferent or efferent axons with trains of pulses is unknown. However, since the microelectrode tips would most likely be near afferent or interneuronal axons rather than efferent cell bodies or axons, any effect of reduced thresholds would probably be most significant on afferent or interneuronal axons.

Given the increased afferent axon density in the dorsal horn and intermediate gray matter compared to the ventral horn (Brown, 1981), one might predict that the threshold for detecting dorsal rootlet action potentials would be lower with stimulating electrodes located in these more dorsal regions. However, this was not observed. Differences in the dorsal rootlet ENG detection thresholds were observed with different electrodes, but did not seem to correlate with electrode depth. Also, as mentioned previously, no relationship between the stimulating electrode depth and the dorsal rootlet ENG threshold at different rostrocaudal locations was found. This implies that electrodes in the dorsal horn and ventral horn are equally likely to elicit compound dorsal rootlet action potentials at various rostrocaudal locations. The fact that in some cases, larger stimulation amplitudes were required to elicit compound action potentials in dorsal rootlets farther from the site of ISMS suggests that the density of axon collaterals decreases (particularly in the rostral direction).

As the distance between the ISMS electrode and recording electrodes on a dorsal rootlets increased, a rostrocaudal asymmetry in the dorsal rootlet ENG threshold was observed. The reason for this asymmetry is unclear, although PAD mediated by centrally controlled presynaptic inhibition could conceivably cause these results. Lomeli et al. (1998) have demonstrated that lumbar segmental and ascending collaterals of the same primary afferent exhibit differing amounts of PAD as a result of centrally controlled presynaptic inhibition of the interneurons controlling PAD. Asymmetries between rostrally and caudally projecting collaterals have also been described in terms of conduction velocities (Wall, 1994), but any relationship between this and the results observed in this study are unknown.

In addition to comparing dorsal root ENG and hindlimb muscle EMG onset latency differences, the progression of EMG onset latencies themselves provide insight into the recruitment of neurons with ISMS. At high stimulation amplitudes (>100 μA), the onset latency was generally on the order of 2-4 ms. However, at lower amplitudes, EMG onset latencies increased dramatically up to 14 ms. The maximum distance between intraspinal stimulating and EMG recording electrodes was on the order of 250 mm. Assuming the lowest reported cat MN conduction velocity of 60 mm/ms (Matthews, 1972), a delay of 0.5 ms at the neuromuscular junction and a further delay of 0.5 ms for muscle fiber action potentials to be detected by EMG electrodes, only 5 ms are accounted for, providing an upper limit on what could be considered "direct" activation of MNs. Since EMG responses were frequently detected with onset latencies greater than 5 ms, these responses must have been generated by the synaptic activation of MNs by afferent axons or axons of interneurons. This pattern of presumed synaptic activation of MNs occurred frequently when ISMS amplitudes were below 100 μA. In cases where EMG onset latencies were very long (8-14 ms), polysynaptic transmission, and possibly slowly conducting axons of afferents and interneurons are implicated. Since muscle fibers themselves conduct action potentials slowly (approximately 4 mm/ms), a potential source of error in estimating latencies was the distance between the motor endplate zone and the nearest EMG electrode. However, the bipolar electrode configuration used is sensitive to potential changes occurring at a distance and given that EMG was reliably detected at minimal latencies of 2-4 ms in all recorded muscles at high stimulation amplitudes, the latency estimates were probably no more than 2 ms too large. These minima were assumed to represent direct motoneuronal activation.

One issue surrounding the use of ISMS for neuroprostheses is that of sensory perception. Activation of afferents and ascending tracts could potentially cause discomfort or pain. While we did not examine what kinds of afferents were activated in our experiments, pain induced by ISMS was not observed in awake spinally intact cats with chronically implanted electrodes in the ventral horn (Mushahwar et al., 2000). The only human studies using ISMS, in which micturition was the desired outcome, excluded subjects with sensation below the level of spinal cord injury (Nashold et al., 1972, 1977). Given that the afferents activated by ISMS in intermediate and ventral gray matter are most likely large muscle afferents, the sensations evoked in incomplete spinal cord injury subjects would likely be similar to those evoked by peripheral nerve neuroprostheses such as the BIONic WalkAid (Weber et al., 2005).

In previous ISMS studies with electrodes targeted to achieve functionally relevant hindlimb movements, stimulation amplitudes were generally in the range of 50-250 μA (Mushahwar et al., 2000). In our study, electrodes targeted the gray matter and were not positioned to elicit specific hindlimb movements, although the most ventral electrodes were near the regions of the spinal cord where such movements would be expected. Under these conditions and stimulation amplitudes in the range of 50-250 μA most of the recorded muscles exhibited detectable activity. In animal R3, the three deepest electrodes co-activated the antagonistic pair LG and TA regardless of stimulation amplitude. In animal R2, at 100 μA, all of the ISMS electrodes caused contractions of LG, MG, SMa, BFp and BFa; the entire set of recorded muscles. A similar pattern was observed in the other animals where recordable EMG activity was detected in nearly every muscle at stimulation amplitudes high enough to generate functional movement. Single-joint movements and multi-joint synergies (Mushahwar et al., 2000) as well as locomotor patterns (Guevremont et al., 2003) can be achieved with carefully positioned ISMS electrodes in the ventral horn. However, the present results suggest that stimulation at many locations in the gray matter produce widespread activation of many hindlimb muscles.

This experimental evidence has corroborated previous work, both experimental and theoretical, suggesting that within the CNS, axons are activated at lower thresholds than cell bodies. However, perhaps surprisingly, it was found that afferent axons were nearly always activated antidromically at lower thresholds than the large MN axons even when the stimulating electrodes were deep within the ventral horn. It was also demonstrated that the spread of activity within the spinal cord mediated by these afferent fibers can extend for at least 17 mm rostrally and caudally, covering the entire lumbosacral enlargement. This spread of activity activates large numbers of muscles and may explain some of the observations that led to the movement primitives hypothesis. Our results also help explain how ISMS activates large populations of neurons distributed over several spinal segments, and should be taken into account in the interpretation of results obtained with microstimulation within the CNS.

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Chapter 5

High-frequency blocking of the pudendal nerve using transcutaneously coupled electrical stimulation*

> *"And now for something completely different."*
>
> \- Monty Python

5.1 Introduction

The act of micturition normally involves the coordinated activation of the detrusor (bladder wall) muscle and relaxation of the external urethral sphincter (EUS) muscle. During the maintenance of continence, the detrusor remains relaxed while EUS contractions help prevent leakage, while during micturition, the pattern of activity reverses and the EUS relaxes while the detrusor contracts, resulting in the expulsion of urine. Injury and disease involving the nervous system, including spinal cord injury (SCI) and multiple sclerosis can disrupt this normal control scheme resulting in an impairment of storage, voiding, or both.

After SCI, detrusor-sphincter dyssynergia (DSD) is a frequently observed phenomenon in which the EUS contracts reflexively in response to contractions of the bladder (Kaplan et al., 1991; Weld and

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* A version of this chapter has been submitted for publication.

Dmochowski, 2000; Yalla et al., 1977). DSD therefore prevents normal voiding and if left untreated can lead to high intravesical pressures and renal failure. Many techniques have been proposed to overcome the problem of DSD (Gaunt and Prochazka, 2006), but the current best practices involve pharmacological treatment to reduce hyperreflexive bladder contractions combined with clean intermittent or suprapubic catheterization to drain the bladder. Despite these treatments, urinary tract infections and other urinary related complications remain the leading cause of hospitalization after SCI (Middleton et al., 2004). There remains a strong impetus to develop better solutions to the problem of bladder control after SCI.

One relatively recent approach to overcoming DSD after SCI involves the use of high-frequency stimulation (HFS) of the pudendal nerve to block action potential propagation in the nerve, thus unwanted EUS activity (Bhadra et al., 2006; Tai et al., 2004, 2005c). The pudendal nerve contains the motor axons to the EUS as well as other efferent and afferent fibers innervating the external anal sphincter, other perineal musculature and the genitalia (Martin et al., 1974). HFS has been investigated sporadically over the past 100 years and has been shown to block the propagation of action potentials locally in peripheral nerve. Kilgore and Bhadra Kilgore and Bhadra (2004) provide a thorough summary of the history of HFS and the various and inconsistent ways it has been implemented and reported. Part of the reason for the confusion in this area of research is that the term "high-frequency stimulation" has been used to describe stimulation frequencies from less than 100 Hz all the way up to 50 kHz. In addition, a complete theoretical understanding of the mechanism of action of HFS has yet to be achieved. However, recent work has done much to address this confusion and to propose theoretical models to explain the mechanism of high-frequency (HF) blocking (Bhadra and Kilgore, 2005; Bhadra et al., 2007; Kilgore and Bhadra, 2004; Tai et al., 2005a,b; Zhang et al., 2006a,b). In addition to reducing unwanted EUS contractions (Abdel-Gawad et al., 2001; Bhadra et al., 2006; Ishigooka et al., 1994; Shaker et al., 1998; Tai et al., 2004, 2005c), there have been reports of using HFS to control motor axon recruitment order during electrical stimulation (Solomonow et al., 1983; Zhou et al., 1987) and to suppress tinnitus (Rubinstein et al., 2003).

Recently, a transcutaneous stimulation delivery system, referred to as the stimulus router system (SRS), that passes current directly through the skin rather than relying on inductive coupling across the skin was described (Gan et al., 2007). An external stimulator connected to surface electrodes applied to the skin was used to transmit stimulation pulses to deep-lying nerves using completely passive implanted electrodes. Given the potential usefulness of HFS in blocking unwanted EUS contractions, the primary aim of this study was to examine whether this new stimulus delivery system could transmit HF waveforms sufficient to produce a functional conduction block of the pudendal nerve.

5.2 Methods

Four adult male (#1, #2, #3, #6) and two adult female (#4, #5) cats (age: 1.5 - 4 yrs, weight: 2.7 – 6.5 kg) were used to test the hypothesis that the SRS is an effective means to deliver HF waveforms to the pudendal nerve in order to block action potential propagation. Acute experiments were performed on all animals except #6. In animal #6 a chronic implant was performed to evaluate the performance of HFS at various time points. All experiments were done with the approval of the University of Alberta Animal Care and Use Committee.

5.2.1 Surgical Procedures

The animals were pre-operatively medicated with acepromazine (0.1 - 0.25 mg/kg sc), glycopyrrolate (0.01 mg/kg sc) and buprenorphine (0.01 mg/kg sc) or hydromorphone (0.1 mg/kg sc) and then anesthetized with a mixture of isoflurane (2-3% in carbogen, flow rate 2 L/min). For the acute experiments, the trachea was cannulated and connected to a closed loop anesthetic system that monitored respiration rate and pressure and ventilated the animal if necessary. For the chronic implant, anesthesia was delivered via a pediatric endotracheal tube. The cephalic vein was catheterized to allow administration of fluids and drugs and a saline drip was delivered throughout the procedure. Body temperature was maintained using a warm water heating pad and the heart rate and SpO_2 were monitored throughout.

For the acute experiments, the bladder was exposed through a midline abdominal incision and catheterized at the dome with a 5 Fr. catheter to allow intravesical pressure measurement as well as the addition and withdrawal of fluid. A purse-string suture was placed around the catheter to prevent leakage. The abdomen was closed in layers with the catheter emerging percutaneously. For the chronic implant, the end of a custom-made silicone catheter was inserted into the dome of the bladder through a puncture hole created with a 16G hypodermic needle and a silk purse-string suture was secured around the catheter. A retaining disk on the catheter was sutured to the bladder wall to ensure that the catheter remained fixed in place. The abdominal musculature was closed with 3-0 Vicryl suture with the catheter emerging at the rostral end. A trocar was used to draw the catheter subcutaneously to the cat's head. The abdominal skin was sutured closed with 3-0 Prolene suture. Four stainless steel screws were attached to the skull through small incisions which were then sutured closed. The screw heads as well as a Luer fitting attached to the bladder catheter were embedded in a dental acrylic cap. This provided a secure platform for long-term access to the bladder catheter connector while maintaining skin health.

The pudendal nerves were exposed bilaterally by incisions lateral to the base of the tail and by blunt dissection through the tissue of the ischio-rectal fossa. For the acute experiments, two bipolar platinum hook electrodes or stainless steel electrode nerve cuffs were placed on exposed portions of the pudendal nerve unilaterally. Both the proximally and distally placed electrodes captured the caudal rectal and deep perineal branches of the pudendal nerve (Martin et al., 1974). In the case of animal #3, a laminectomy was performed and a bipolar nerve cuff was placed on the S2 spinal nerve root intradurally instead of on the proximal pudendal nerve. For the chronic implant, custom made implants consisting of three SRSs for the left and right hand sides were implanted (see Figure 5.1 for a schematic and description of the implant). The nerve cuff used in the chronic implant consisted of a 10 mm length of split silicone tubing containing three contacts. The proximal contact was used to deliver low-frequency stimulus waveforms while the distal two electrodes were used in a bipolar configuration to deliver HF waveforms. The stimulating electrode was separated from the blocking electrodes by 4 mm and the bipolar blocking electrodes had an interelectrode spacing of 2 mm. This particular nerve cuff length and electrode configuration was chosen to fit the available length of pudendal nerve in a chronic implant while still containing the required electrodes.

At the end of the chronic implant, the cat was given ketoprofen (1 mg/kg sc) and hydromorphone

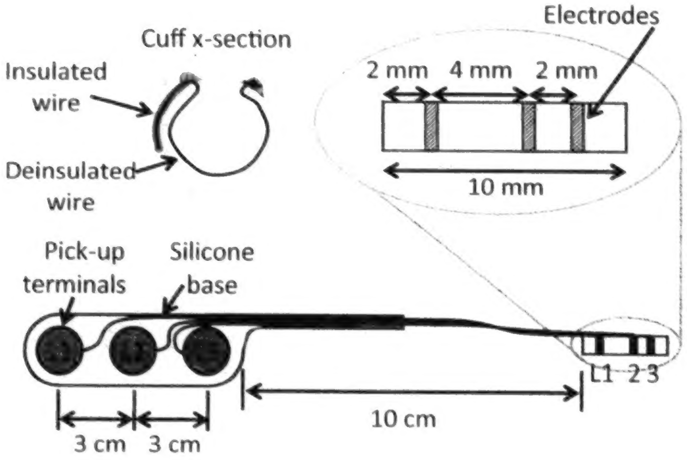

Figure 5.1: *Schematic of the left-hand side chronic implant*

The nerve cuffs were constructed using Silastic tubing (1.02 mm ID, 2.16 mm OD) and Cooner AS636 stainless steel wire. The Silastic tubes were sliced longitudinally and a section of deinsulated wire was inserted through one edge of the tube and exited at the opposite edge. At both the entry and exit from the tubing, a bead of silicone was applied to both insulate the wire and secure it to the tubing. Three such electrodes were placed in each cuff as shown. The SRS pick-up terminals were made from stainless steel disks 1.5 cm in diameter and were embedded in a custom made silicone base. The disks were separated by 3 cm and a 1 cm diameter window was cut in the silicone base to expose the metal disk. The array of pick-up electrodes was positioned in the subcutaneous space near the lumbar vertebrae. The electrodes in the nerve cuffs were connected to SRS pick-up electrodes via Cooner AS636 wire.

(0.07 mg/kg sc) sufficient to maintain a somnolent state. During post-operative recovery the cat was kept warm in a heated cage. Analgesia was maintained by giving two or three additional doses of ketoprofen and/or hydromorphone at 8-hour intervals. Ampicillin was administered for 4 days after surgery, followed by amoxicillin (62.5 mg tablets, 2/day) for 6 additional days. At the termination of all acute experiments the animals were euthanized with an overdose of pentobarbital sodium.

5.2.2 Stimulus Router System

The SRS used throughout these experiments provided a means to transmit electrical current from surface electrodes through the skin and then to the pudendal nerve via an implanted lead wire. The SRS consists of several components: the external stimulator, surface electrodes, and lead wires with

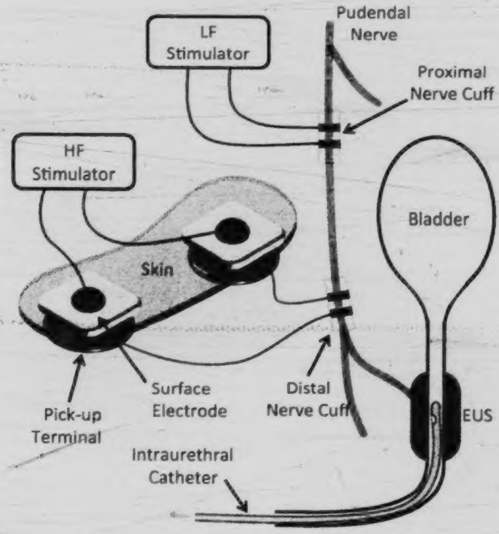

Figure 5.2: *Schematic of the SRS and the setup for the acute experiments used to test HF blocking of the pudendal nerve*

The two electrodes in the distal "blocking" cuff were each connected via insulated wire to a "pick-up terminal" made from a stainless steel metal disk (1-1.5 cm diameter) placed under the skin. Adhesive gel electrodes were applied on the skin directly over the pick-up electrodes and connected to a stimulator. The proximal nerve cuff was connected directly to a stimulator to generate LF pulse trains. An intraurethral catheter was positioned with its side-port in the region of the EUS to measure intraurethral pressure during LFS and HFS.

pick-up and stimulation terminals. Adhesive gel electrodes placed on the skin were positioned directly over subcutaneously placed pick-up terminals which were connected via insulated lead wires to stimulation terminals (hook electrodes or nerve cuff electrodes). When current was passed between pairs of surface electrodes, a portion of the current was captured by the implanted lead and routed to the nerve cuff to stimulate the pudendal nerve. Figure 5.2 shows a schematic representation of this system and the setup for the acute experiments in this study. In this system, the ratio of internal current (current flowing to the nerve) to the external current (total current delivered by the stimulator) has been shown to be in the range of 0.10 - 0.18 (Gan et al., 2007).

5.2.3 Stimulation Procedures

Two types of stimulation were used in these experiments: direct stimulation and SRS stimulation. In direct stimulation, the bipolar electrodes of the hook electrode or nerve cuff, placed on the pudendal nerve, were connected directly to a pulse generator. When SRS stimulation was used, the distally placed hook electrodes or nerve cuff electrodes were connected as shown in Figure 5.2. The adhesive gel surface electrodes (Kendall Soft-E H69P, Kendall-LTP, Chicopee, MA) were applied to the skin after it was carefully shaved and cleaned with alcohol. The pick-up electrodes were positioned subcutaneously near the lumbar vertebrae.

Low-frequency (LF) (~15-30 Hz) direct stimulation via proximally placed hook or nerve cuff electrodes on the pudendal nerve was used to elicit contractions of the EUS. In one case (#3), contractions of the EUS were generated by stimulation of the S2 spinal nerve root intradurally. LF pulse trains were generated using either a voltage-controlled Grass SD9 stimulator (Grass Technologies, West Warwick, RI) or a current-controlled Neurolog stimulator (Digitimer Ltd., Welwyn Garden City, UK) using modules NL304 (period generator), NL403 (delay-width), NL510 (pulse buffer) and NL800 (stimulus isolator). HF pulse trains were generated using the Neurolog system (animals #1 and #2) or a custom-built constant-current stimulator (animals #3 - #6). Both the Grass SD9 and Neurolog stimulators generated monophasic pulses only, while the custom-built stimulator was used to generate biphasic waveforms.

In the chronic implant (animal #6), both LF and HF pulse trains were delivered via the SRS implants. The response to HFS was normally evaluated with the animal anesthetized with isoflurane. Prior to a stimulation session, the skin over each pick-up electrode was shaved and cleaned and adhesive gel surface electrodes (Kendall H69P) were applied. LF pulse trains were delivered using the Grass SD9 stimulator through the most proximal pudendal nerve electrode (L1 – see Figure 5.1). An additional adhesive gel electrode (Kendall H49P) was positioned over the base of the tail to act as the indifferent (anode) electrode during LF monopolar stimulation. The Grass SD9 stimulator provided voltage-controlled monophasic pulses 200 μs in duration. HF waveforms were current-controlled charge-balanced sinusoids applied through the L2 and L3 surface electrodes. Six evaluation sessions were performed over the course of 6.5 months.

A typical HFS trial consisted of 10 seconds of LF stimulation (LFS) delivered through the proximal electrode followed by 5 seconds of LFS + HFS and a final 10 seconds of LFS only. From trial to trial both the HFS amplitude and frequency were varied. Typically, the HFS parameters ranged from 1 – 10 kHz and 1 – 10 mA. In all experiments, the bipolar HF electrodes were separated by 2 mm and were 0.5 – 1 mm wide.

5.2.4 Pressure Measurement

Bladder and intraurethral pressures were measured using an implanted bladder catheter and a closed-end tomcat catheter (Kendall 3.5 Fr), respectively. Each catheter was connected to a Neurolog NL108-D4/10 pressure dome and NL108T4 isolated pressure transducer (Digitimer Ltd., Welwyn Garden City UK). The urethral catheter was modified to block the distal of the two side ports at the tip of the catheter. The urethral catheter was also connected to an infusion pump (Pump 22, Harvard Apparatus, Saint Laurent, Quebec, Canada) and during intraurethral pressure measurements, sterile saline was infused at a rate of 0.2 or 1.0 mL/min. This method of measuring urethral pressure was first described by Brown and Wickham (1969). The side port of the urethral catheter was positioned in the region of the EUS (typically 4-6 cm from the tip of the urethral meatus). Both the bladder and urethral pressure signals were displayed on an oscilloscope and the data were sampled at a rate of 100 samples per second using a CED 1401 Laboratory Interface and Signal v3 software (Cambridge Electronic Design Ltd., Cambridge, UK).

5.2.5 Data Analysis

An automated analysis of the intraurethral pressure traces was performed using software written in Matlab R2007a (The Mathworks, Natick, MA) to extract three measures of performance in the HFS trials. Figure 5.3 shows an example of a HFS blocking protocol and the resulting intraurethral pressure trace. From this trace, the baseline pressure (22 mmHg), pre-block pressure (118 mmHg), peak onset pressure (142 mmHg), minimum block pressure (32 mmHg) and peak recovery pressure (129 mmHg) were found. These values were used to calculate the following three measures: block percent, onset percent and recovery percent. In the example of Figure 5.3 where the stimulation amplitude was 8 mA and the stimulation frequency was 8 kHz, the block percent was 90%, the onset percent was 25% and the recovery percent was 110%. Block percent, onset percent and recovery percent were calculated as follows:

$$Block\,\% = \left(\frac{P_{PreBlockMax} - P_{Block}}{P_{PreBlockMax} - P_{Baseline}}\right) \times 100$$

$$Onset\,\% = \left(\frac{P_{PeakOnset} - P_{PreBlockMax}}{P_{PreBlockMax} - P_{Baseline}}\right) \times 100$$

$$Recovery\,\% = \left(\frac{P_{RecoveryMax} - P_{Baseline}}{P_{PreBlockMax} - P_{Baseline}}\right) \times 100$$

These three measures were selected to provide quantitative information regarding the completeness of the block, the size of the onset response and the effect of the block on subsequent neural and muscular function.

Statistical analysis was performed using Matlab R2007a and SigmaStat v3.5 (Systat Software, Inc., San Jose, CA). Kruskal-Wallis one way analysis of variance on ranks was used to test for differences between multiple groups. Student's t-test was used to test for the difference in means of two groups. The text indicates which test was used in specific instances. Simple linear regression was also used to examine relationships between variables and ANOVA was used to test for significance in these cases. In all cases results are reported as significant with $p < 0.05$. At various points, the data are divided into two groups: the first group includes all collected data and the second group includes only data where the block percentage was > 80%. This second group was selected to provide an indication of those trials in which "near complete" blocking was achieved and where the blocking would likely lead to functionally relevant results. Grouped data are reported in the text as the mean ± standard deviation.

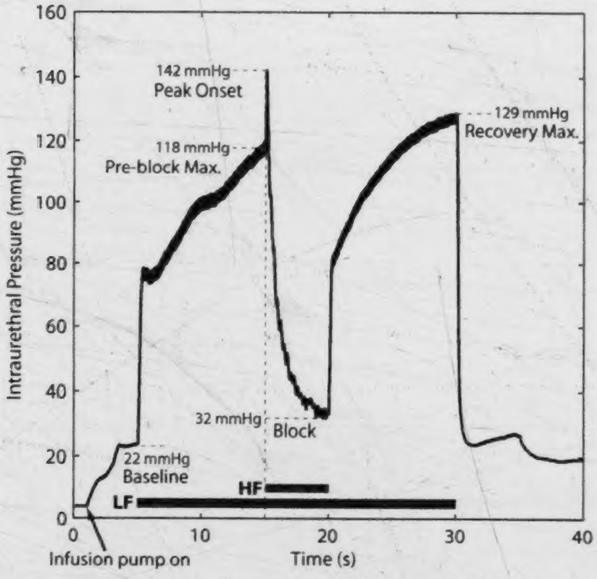

Figure 5.3: *Example of pudendal nerve block and calculated parameters*

Example of reduction in intraurethral pressure caused by HFS and the automatically calculated parameters used to quantify specific measures of the block. Baseline pressure (22 mmHg) was calculated as the average of the pressure for the 2 seconds prior to the onset of LFS. Pre-block pressure (118 mmHg) was calculated as the average of the pressure for the 2 seconds prior to the onset of HFS. Onset pressure (142 mmHg) was the maximum pressure during the 5 seconds of HFS. Minimum block pressure (32 mmHg) was the minimum pressure during the 5 seconds of HFS. Recovery pressure (129 mmHg) was the maximum pressure during the 10 seconds of LFS following the cessation of HFS. The black bar labeled "LF" indicates the period of time when the LFS was on. The gray bar labeled "HF" indicates the period of time when the HFS was on. In this example the LF waveforms were voltage controlled monophasic pulses (duration = 200 μs, amplitude = 25 V, frequency = 20 Hz) delivered through the L1 electrode. The HF waveforms were current-controlled continuous sinusoids (amplitude = 8 mA, frequency = 8 kHz) delivered through electrodes L2 (cathode) and L3 (anode). Note that the rise in intraurethral pressure during the first 3 seconds of the trace was caused by the infusion pump turning on.

5.3 Results

The primary aim of these experiments was to determine whether or not HF waveforms could be transmitted by the SRS to the pudendal nerve to block ongoing EUS contractions. In the first group of three animals, the experimental goal was to determine the feasibility of the approach and in the last three animals the experimental goals were to more thoroughly examine the details of the nerve blocking including efficacy, onset, and recovery and the stimulation parameters required to achieve blocking. Figure 5.4 shows characteristic examples of the HF blocking achieved in each animal. Stimulation amplitudes are reported as the current delivered to the external surface electrodes. The amount of current transmitted through the skin was not measured, but using a conservative estimate based on previous work, approximately 10% of the external delivered current would be transmitted to the nerve (Gan et al., 2007). Although not thoroughly examined, HFS appeared to have no effect on skin health in either the acute or chronic experiments.

5.3.1 Block Efficiency

In 5 of 6 animals complete or near-complete blocking of proximally-generated EUS contractions was achieved. In these 5 animals, the maximum block percentage observed was 107% ± 11% (range: 90% to 120%). In the other animal (#5), the maximum blocking percentage obtained was 34%. Although HFS did occasionally lead to reductions in intraurethral pressure in this animal, the pressure decrease did not begin until well into the HFS train leading to the generally poor responses observed. Table 5.1 summarizes the number of trials performed in each animal and how often a block percentage > 80% was achieved. In the first three animals (#1, #2 and #3), the range of parameters was not thoroughly explored which accounts for the low number of trials. Figure 5.5 shows histograms of the block, onset and recovery percentages for all of the animals. When the minimum HFS amplitudes required to produce 80% blocking at each tested frequency were grouped for all experimental sessions in all animals, a positive linear relationship between the HFS frequency and HFS amplitude was found over the tested frequency range (1-10 kHz). Although the coefficient of determination (R^2) was only 0.24 for the grouped data, the relationship was significant ($p<0.001$) and had a slope of 0.43 mA/kHz

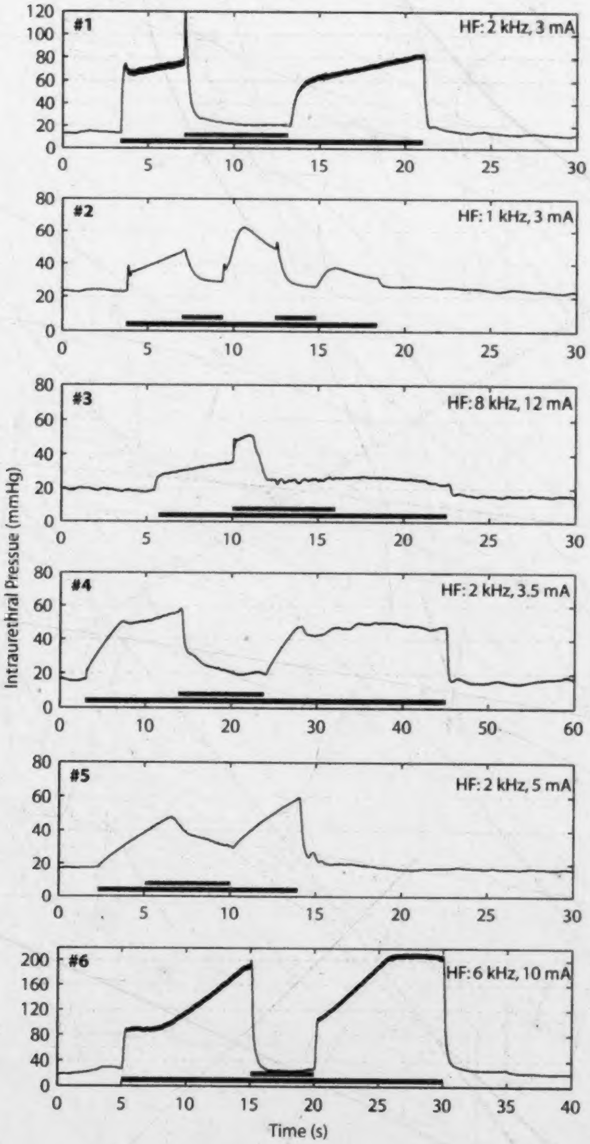

Figure 5.4: *Example of HF blocking in each animal*

The HFS parameters are listed in each panel. These figures were selected to represent an average trial from these particular animals and do not show the best result. Substantial variability can be seen in the responses seen in each animal.

Table 5.1: *Summary of the trials performed in each animal.*

The sex of each animal is listed in the second column. The third column displays the total number of trials that elicited > 80% blocking over the total number of trials performed. Since many stimulation parameter combinations were tested more than once, the fourth column displays the total number of unique stimulation parameter combinations tested that elicited > 80% blocking over the total number of unique stimulation parameters tested.

ID	Male / Female	Total > 80% Block	Unique Parameters > 80% Block
#1	M	5/8	3/5
#2	M	8/13	6/9
#3	F	9/18	4/8
#4	M	16/37	14/34
#5	F	0/12	0/11
#6	M	107/214	55/78

with a y-intercept of 3.7 mA. This linear relationship indicates that to reach the threshold for 80% blocking, the HFS amplitude must be increased as the HFS frequency is increased.

5.3.2 Onset Response

At the onset of HFS, a transient increase in intraurethral pressure, δP, was nearly always observed. Previous modeling work has shown that this increase is caused by an initial period of nerve activation elicited by HFS (Elbasiouny and Mushahwar, 2007; Kilgore and Bhadra, 2004). Across all trials, δP was 23.6 mmHg ± 19.8 mmHg (range: -0.2 mmHg to 81.6 mmHg) representing an increase over the pre-block pressure of 27.4 ± 23.6 % (range -0.3% to 122.4%). Several negative values of δP were obtained because of the method of calculating the pre-block pressure. For example, if the intraurethral pressure was decreasing in the two seconds prior to the onset of HFS, it was possible for the average pressure during this window to be higher than the maximum increase in pressure at the onset of HFS, leading to a negative value of δP. In animal #6 in which the widest stimulation parameter range was examined, there was a negative linear relationship between δP and both the HFS frequency ($p<0.01$) and amplitude ($p<0.001$) across all trials. However, the coefficient of determination was 0.036 and 0.09 for the relationships respectively indicating that HFS frequency and HFS amplitude had only very weak predictive value for δP. When the data from animal #6 was considered on a session by session basis, several high R^2 values were found, but changed substantially between sessions. For

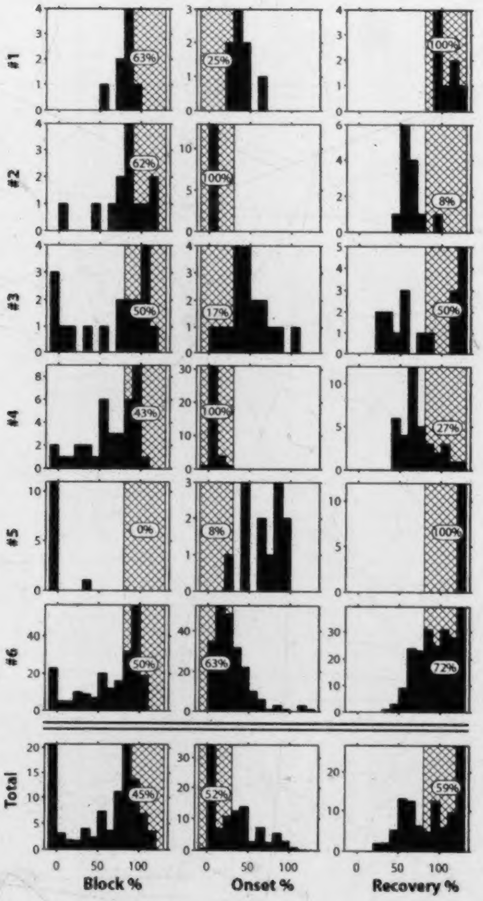

Figure 5.5: *Histograms showing block percent, onset percent and recovery percent for each animal*

Each row shows the data for a particular animal while the bottom row shows the combined data for all animals. For the individual animals, the y-axis shows the number of trials that fell into each bin. In all cases, the bin widths are 10% and range from 0% to 120%. To the left of the 0 - 10% bin is an additional bin that includes all the trials in which activation rather than blockade occurred, while to the right of the 110-120% bin is an additional bin that includes the number of trials greater than 120%. For the combined data in the last row, the y-axis shows the average percentage of trials that fell within each bin with the data from each animal weighted equally. This was done so that the data from animal #6 did not dominate the combined data simply because of the large number of trials. In each panel the hatched area indicates the desired response: > 80% for the block percentage, < 30% for the onset percentage and > 80% for the recovery percentage. In each panel, the value displayed over the hatched area indicates the percentage of trials that were within the range covered by the hatched window.

example, during the second recording session R^2 for the regression between HFS frequency and δP was 0.54 ($p < 0.001$), while it was not significant for the HFS amplitude. During the third recording session however, the regression between HFS frequency and onset amplitude was not significant, while R^2 was 0.75 for the regression between HFS amplitude and δP ($p < 0.001$). Figure 5.5 shows histograms of δP expressed as onset percentages for each animal. There was a significant difference in the onset percentages between animals (Kruskal-Wallis, $p < 0.001$). In all cases where a significant relationship was present between δP and HFS frequency or amplitude, δP decreased with increasing HFS frequency or amplitude. This was true for the grouped data for each animal as well as for the individual session data for animal #6.

5.3.3 Nerve Recovery

The responsiveness of the nerve after the termination of HFS is important in determining whether HFS has a long-lasting effect on axonal conduction. In these experiments, this was measured by examining the changes in intraurethral pressure after termination of HFS while proximal LFS was still being delivered. Across all experiments, the mean recovery was 98% ± 40% (range: 22% to 267%) of the maximum pre-block pressure while for those trials where the block percentage was > 80%, the mean maximum recovery was 83% ± 24% (range: 22% to 130%) of the pre-block pressure. Figure 5.5 shows histograms of the recovery percentage for each of the animals. There was a significant linear relationship between the maximum pre-block pressure and the maximum recovery pressure for animals #1, #2, #3, #4, and #6 with the slope being 0.98 ± 0.26 (range: 0.54 to 1.20). The coefficient of determination for these regressions was 0.60 ± .26 (range: 0.17 to 0.82). For these animals, the time to reach 90% of the maximum pressure for the grouped data was 3.26 ± 3.06 s (range: 0.01 to 16.9).

5.3.4 Monopolar - Bipolar Comparison

In a number of instances, a monopolar electrode configuration was used to deliver HFS as opposed to the usual bipolar configuration. The monopolar configuration produced a significantly smaller block percentage than the bipolar configuration at the same amplitude and frequency (paired t-test, p

= 0.019). In some cases the bipolar configuration elicited a near complete block while the monopolar configuration elicited an increase in intraurethral pressure (example: bipolar block 97%, monopolar block -22%) while in other cases, the percentages were nearly equal (example: bipolar block 100%, monopolar block 94%).

5.3.5 Awake Animal Tests

In the chronically implanted animal, the responses to LFS and HFS were evaluated in the awake state during one session. Intraurethral pressures could not be measured because of the difficulty in placing the urethral catheter in an awake animal. During the LFS trial (20 Hz, 1.8 mA), there were no visible responses or aversive reactions. These stimulation parameters were chosen as they had previously elicited large increases in intraurethral pressure. At a constant frequency of 6 kHz (demonstrated to elicit blocking during anesthetized trials), the stimulation amplitude was varied from 2 to 10 mA. The first movement of the animal in response to HFS occurred at 5 mA. At stimulation amplitudes greater than 5 mA, there was a mild aversive response only at the onset of stimulation consisting of a small twitch-like reaction involving the whole body. No vocalization nor attempts to move away occurred at any stimulation amplitude. A 10 s long train of HFS at 10 mA caused no further response than a 1 s long train of HFS at 7 mA.

5.4 Discussion

The results presented in this paper show that HF waveforms can be passed through the skin using the SRS and delivered to the pudendal nerve to completely block action potential propagation in the nerve induced by proximal LFS. Blocking of the pudendal nerve was evaluated by measuring the change in intraurethral pressure as measured by an intraurethral infusion catheter positioned in the vicinity of the EUS. Complete (ie. > 80%) blocking of the pudendal nerve was achieved in five of the six animals tested using the SRS indicating that this is an effective method of delivering HF waveforms trains. In addition, HFS using the SRS behaved in a similar manner to previously published studies of HF blocking when direct connections were made from the electrical stimulator to the stimulation electrodes.

Previous studies of HFS have found that higher stimulation frequencies require higher stimulation amplitudes to block peripheral nerve and that these values are linearly related (Bhadra et al., 2006; Bhadra and Kilgore, 2005). The same studies also found that the amplitude of the onset response was minimized at higher stimulation frequencies and amplitudes. Both of these relationships were found in the present experiments when HF waveforms were delivered to the pudendal nerve using the SRS. Other work on HFS has indicated a wide range of minimal frequencies that produce an effective block. One study (Shaker et al., 1998) reported that 600 Hz could elicit a block of a sacral root while other studies have suggested minimum blocking frequencies of 1 kHz (Bhadra et al., 2006) and 4 kHz (Tai et al., 2004, 2005c) to block action potential propagation in the pudendal nerve. We did occasionally observe large reductions in intraurethral pressure during HFS at frequencies < 1 kHz, however, it was more common for the minimum frequency to be in the range of 1-3 kHz. Modeling studies of HFS suggest that the minimum frequency required to block axons is in the range 3 - 15 kHz (Elbasiouny and Mushahwar, 2007; Tai et al., 2005b; Williamson and Andrews, 2005; Zhang et al., 2006b), although the specific model used can have a large influence on these estimates. The modeling studies all used models that were developed to predict activation of axons with low-frequency pulse trains and may not necessarily be well suited to predict all the details of HF blocking.

In the present experiments it was demonstrated that HFS could be tolerated by an awake animal. Although there were mild aversive responses at the onset of stimulation, suggesting startle or transient discomfort, continued HF stimulation was well tolerated. The issue of aversive responses is particularly important for pudendal nerve stimulation as this nerve contains both motor and sensory fibers and it is possible that stimulation of the pudendal nerve could lead to pain. Although there was evidently a sensory volley transmitted through the pudendal nerve coincident with the onset of HFS, there was no evidence of continued unpleasant sensations. This suggests that clinical implementation of HFS may be acceptable even when it elicits large onset responses. While it would be desirable to eliminate any and all unwanted side effects of electrical stimulation of nerves, this is often impossible given the many types of sensory axons in peripheral nerves. Movement of the leg is often an unwanted side effect resulting from activation of efferent fibers in the sacral roots when stimulating them to elicit bladder contractions (Brindley, 1994). Yet these are the most successful neuroprostheses for bladder control.

A number of trials comparing the effectiveness of monopolar and bipolar HFS were tested. While monopolar stimulation was capable of blocking the pudendal nerve, it was not as reliable as bipolar stimulation and generally required higher stimulation amplitudes. However, further characterization of the full range of possible parameters using monopolar stimulation may show that functionally relevant and consistent HF blocking may be achievable. Given the simplicity of a monopolar SRS as compared to bipolar or even tripolar systems, there may be practical benefits for pursing HF block using a monopolar stimulation electrode.

Although complete blocking of the pudendal nerve was achieved in 5 of the 6 animals tested, there was a large amount of variability in the quality of the blocking and in the most effective stimulus parameters. During the multiple recording sessions in animal #6, which occurred over 6 months, two very different parameter ranges were observed for eliciting a complete block. The higher-frequency range generally elicited effective blocking only when the stimulation frequency was greater than 4 kHz and the amplitude was greater than 7 mA. The lower-frequency range produced effective blocking when the stimulation frequency was 2-4 kHz and the amplitude was greater than 2 mA. It is important to note that on those days where the lower-frequency range was effective, no combination of stimulation frequency and amplitude could be found above 4 kHz that elicited blocking. Initially, the higher-frequency range was the most effective, but on post-implant days 127 and 176, the lower-frequency range was most effective. During the last test (day 230), the higher-frequency range was again the most effective. In both the lower and higher-frequency ranges, blocking was complete and occurred rapidly, but when the lower-frequency range was most effective, the recovery was incomplete, whereas when the higher-frequency range was most effective, recovery was generally complete. It is unclear why two independent frequency ranges were observed on different testing days. Since the recovery of intraurethral pressure after the offset of HFS at the lower-frequency range was poor, it is possible that a neural fatigue process with a slow recovery may have contributed to the blocking effect at this lower-frequency range. Despite the inconsistencies, complete blocking was achieved on each tested day.

In one of the experimental animals, HFS was generally ineffective in reducing intraurethral pressures. While the intraurethral pressure usually began to decrease approximately half way through the HFS train, the magnitude of the pressure decrease was small and the pressure decreased slowly. This particular animal in which HFS elicited poor blocking was a female, making the positioning of the catheter more challenging than in the male. Since the quality of intraurethral pressure measurements depends greatly on the specific position of the catheter in the urethra, it is possible that poor catheter placement was a cause of the observed results. It is also possible that the experimental setup including the nerve cuff contact with the pudendal nerve was not optimal in this animal, especially given that complete blocking was achieved in another female cat. Given the proposed mechanisms for HF blocking, including steady-state depolarization due to sodium channel inactivation (Bhadra et al., 2007) and hyperpolarization due to constant potassium channel activation (Zhang et al., 2006a), it is possible that in some animals the effect of isoflurane anesthesia on potassium and sodium channels affects HF blocking (Duch et al., 1998; Nau, 2008). One other possibility that could account for the poor responses in one animal and some of the variability in the other animals is that both LFS and HFS of the pudendal nerve was done unilaterally. Since the EUS is innervated bilaterally, it is possible that proximal stimulation of the pudendal nerve elicited reflex activity in the contralateral pudendal nerve which resulted in EUS activation that was recorded by the intraurethral catheter. Though isoflurane suppresses spinal reflexes, a reflex explanation cannot be ruled out.

One issue that has yet to be addressed in HFS is the potential for nerve damage caused by long term stimulation. No evidence of nerve damage was found in this study as assessed by the functional response of the pudendal nerve to LFS. Over the duration of the chronic implant, LFS thresholds remained stable. With current controlled sinusoidal waveforms, the charge per phase can be expressed as:

$$Q = 1000 \times \frac{A}{\pi \times f}$$

where A is the amplitude in mA, f is the frequency in Hz and Q in the charge in microcoulombs (μC). For stainless steel electrodes, such as the kind used in these experiments, the safe limit of charge per phase has been estimated at 0.4 – 0.8 $\mu C/mm^2$ (Mortimer, 1981). For constant-current sinusoidal waveforms, the highest charge per phase values occur at the lowest frequencies and highest amplitudes. For stimulation at 1 kHz and 10 mA (external), the estimated charge per phase in our experiments would have been 0.2 $\mu C/mm^2$, assuming a 10% capture ratio (1 mA at the pudendal nerve) and an electrode surface area of 1.5 mm^2. Using stimulation parameters that more commonly resulted in complete blocking (eg. 8 kHz, 8 mA), the charge per phase would have been approximately 0.02 $\mu C/mm^2$.

Numerous devices and techniques have been proposed to modify bladder and sphincter function after SCI (Gaunt and Prochazka, 2006). However, very few of these devices have been successful. HF blocking of the pudendal nerve leading to relaxation of the EUS could be a useful way to treat DSD occurring after SCI while reducing the need for catheterization which can be a significant cause of urinary tract infections. Other methods currently available to reduce EUS tone without using catheters or stents are sphincterotomies (Reynard et al., 2003), injections of botulinum toxin (Smith et al., 2005) and less common procedures such as dorsal rhizotomies (Brindley et al., 1986). Each of these procedures has significant disadvantages including the irreversibility of sphincterotomies and dorsal rhizotomies and the repeated treatments required with botulinum toxin injections. HFS of the pudendal nerve would require only a single implant surgery and could be turned on and off at the desired times.

Several aspects of using the SRS to stimulate the pudendal nerve are attractive from a neuroprosthetics perspective. With the stimulus router, the only implanted components are the leads, with their nerve cuff and pick-up terminals. No implanted electronic components or batteries are required. Since all the stimulation electronics are external, a variety of stimulation paradigms using a wide range of stimulation parameters can be explored. For example, no implanted stimulator capable of generating biphasic pulse trains over 1 kHz exists to our knowledge. Also, some studies indicate that different waveform shapes may be better at exciting smaller diameter axons in a peripheral nerve (Bhadra et al., 2002; Fang and Mortimer, 1991). In a normal implanted stimulator, custom electronics would have to be designed to examine these special conditions. With the SRS, an existing implant is capable of transmitting any externally applied waveform. Additionally, maintaining the energy supply to an implanted HF stimulator would be problematic, whereas batteries can easily be replaced in an external device.

Surgical access to the pudendal nerve in humans is not as simple as in the cat, but it is possible to expose the pudendal nerve for the purpose of nerve cuff implantation in humans (Gustafson et al., 2005). Furthermore, a number of other useful responses have been demonstrated with stimulation of the pudendal nerve. Stimulation of the genital branches of the pudendal nerve in humans has been shown to suppress hyperreflexive bladder contractions (Dalmose et al., 2003; Nakamura and Sakurai, 1984; Wheeler et al., 1992) and recent work has shown that stimulation of urethral branches of the pudendal nerve can elicit reflexive bladder contractions in cats (Boggs et al., 2005; Tai et al., 2006) and humans (Gustafson et al., 2004). Taking all of these results together, it may be possible to create a neuroprosthesis where the only implanted component is a lead going to a multipolar nerve cuff on the pudendal nerve that can both inhibit and elicit reflexive bladder contractions as well as inhibit and elicit contractions of the EUS. This range of options represents all of the major actions of the lower urinary tract.

The ultimate goal of HF blocking of EUS contractions is to promote voiding in cases where there are naturally occurring unwanted EUS contractions, or EUS contractions due to proximal stimulation of the pudendal nerve (Boger et al., 2007; Tai et al., 2007). We have shown that it is possible to completely block action potential propagation in the pudendal nerve of anesthetized cats using the SRS. The main objective of future work will be to try to improve the effectiveness of this system in all animals, refine the parameter range and demonstrate the usefulness of HFS in improving micturition. The simplicity of the SRS and the effectiveness of HFS suggest that continued investigation could lead to the development of a device that could be useful in managing DSD.

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Chapter 6

Differential effects of anesthesia on nerve conduction blockade using high-frequency stimulation in two frequency ranges

> *"If your experiment needs statistics, then you ought to have done a better experiment."*
>
> – Ernest Rutherford

6.1 Introduction

High-frequency stimulation (HFS) of peripheral nerves was first shown to block action potential propagation by Cattell and Gerard (1935). Since this time, the technique has been investigated irregularly (reviewed by Kilgore and Bhadra, 2004), but recent interest in potential clinical applications of electrically controlled nerve blockade has led to renewed interest in this phenomenon. The primary clinical application investigated thus far is conduction blockade of the pudendal nerve to abolish contractions of the external urethral sphincter to improve micturition (Abdel-Gawad et al., 2001; Bhadra et al., 2006; Boger et al., 2007; Shaker et al., 1998; Tai et al., 2007).

One of the difficulties in the further development of HFS as a clinically viable technique is an incomplete understanding of the mechanism by which this phenomenon occurs and the factors that affect the characteristic features of high-frequency blocking including the block efficacy itself, the amplitude of the onset response and the recovery after termination of HFS. To date, the majority of the work to clarify these issues has focused on computer modeling to elucidate the block mechanism. One group of papers favours the idea that constant activation of potassium channels near the blocking electrodes leading to axonal hyperpolarization is the underlying cause of high-frequency blocking (Tai et al., 2005a,b; Zhang et al., 2006a), while another set of papers favours the idea that steady-state depolarization of the nerve under the blocking electrodes and constant inactivation of the sodium channels is the mechanism by which action potentials are arrested (Bhadra et al., 2007; Elbasiouny and Mushahwar, 2007; Kilgore and Bhadra, 2004; Williamson and Andrews, 2005). Some of the differences in the results can be accounted for simply by the use of different axon models in different studies. There are also some discrepancies in what is considered the minimal blocking frequency. One modeling study suggested a minimum of 6 to 15 kHz depending on the model used (Zhang et al., 2006a), while another predicted that blocking would occur at all frequencies over 3 kHz (Bhadra et al., 2007). In experimental work, one study reported complete blocking of the pudendal nerve beginning at 1 kHz (Bhadra et al., 2006), while in another a minimal frequency of 6 kHz was needed (Tai et al., 2005c).

To our knowledge, very few, if any studies of HFS have investigated its use in experimental mammalian models in the absence of anesthesia. To investigate this issue, one focus of our experiments with HFS has been to compare the efficacy of HFS in blocking action potential propagation with and without anesthesia. Here, we report on some preliminary observations of the differences we encountered based on results obtained in two animals. Further work is required to confirm these results.

6.2 Methods

Two intact adult male cats (weight: 4 – 5 kg) were used in these experiments. Two distinct experimental preparations were used to examine the effect of anesthesia on HF blocking. The first procedure involved a terminal experiment in which nerve cuffs were placed on the tibial nerve and the effect of

HFS on electrically evoked twitches of triceps surae muscles was recorded using a force transducer before and after decerebration. The second procedure involved a chronic implant of a nerve cuff on the pudendal nerve, followed by complete spinal transection. HFS was delivered through the nerve cuff with and without anesthesia while intraurethral pressure was monitored. These methods provided a way to examine the effects of anesthesia in two independent preparations. All experiments were done with the approval of the University of Alberta Animal Care and Use Committee.

6.2.1 Decerebrate Experiment

One cat was anesthetized with isoflurane in preparation for a decerebration procedure. One carotid artery was ligated and the other was catheterized and connected to a pressure transducer (Neurolog NL108D4/10 dome and NL108T4 isolated pressure transducer, Digitimer Ltd., Welwyn Garden City, UK) to monitor blood pressure. One jugular vein was catheterized to allow administration of drugs. A tracheostomy was performed and an endotracheal cannula was inserted and connected to a closed-loop anesthetic system that ventilated the animal when necessary. Blood pressure, heart rate, SpO_2, and respiration rate were monitored throughout the procedure.

The tibial nerve was exposed on the left hand side and the common peroneal branch was separated by blunt dissection proximally to isolate a length of the tibial nerve approximately 6 cm long. Three bipolar nerve cuffs were place on the tibial nerve with at least 1 cm between each cuff. The incision was closed and the cat was mounted in a stereotaxic frame with ear bars, hip pins and a clamp to fixate the left knee. The calcaneal tuber was detached leaving the Achilles tendon intact. This allowed the tendon to be secured to a force transducer by strong silk suture material. Forces generated by the triceps surae were measured using a custom-built strain gauge apparatus and amplifier and sampled at rate of 100 samples per second using a CED 1401 Laboratory Interface and Signal v3 software (Cambridge Electronic Design Ltd., Cambridge, UK). The tendon was covered with moistened gauze and plastic film to prevent drying for the duration of the procedure. All three nerve cuffs were bipolar with the proximal and distal cuffs having an inter-electrode spacing of 3 mm and the intermediate electrode having an inter-electrode spacing of 4 mm. The proximal and distal electrodes were connected to Grass SD9 stimulators (Grass Technologies, West Warwick, RI). The intermediate blocking electrode was connected to a custom-built stimulator capable of generating high-frequency, constant-current waveforms. The proximal and distal stimulators generated monophasic, constant-voltage rectangular pulses, 300 μs wide at 4 Hz and 1 Hz, respectively. The amplitude of each stimulator was set at the minimum voltage generating a maximal twitch of the triceps surae muscles. Stimulation sessions consisted of 20 seconds of proximal stimulation at 4 Hz with 5 seconds of HFS delivered through the middle nerve cuff beginning 4 seconds after the onset of the proximal stimulus train. During HFS, three stimulation pulses were delivered through the distal nerve cuff to test whether the blockade was occurring at the middle nerve cuff or more distally, for example at the neuromuscular junctions.

After completing stimulation trials under anesthesia, an inter-collicular decerebration was performed after which isoflurane anesthesia was discontinued. Warm water heating blankets and a heating lamp were used to maintain body temperature after decerebration. One hour was allowed for the effects of anesthetic to wear off and for reflexes to recover. Stimulation procedures were then retested. After decerebration, additional trials were conducted where tibial nerve activity was elicited not by proximal electrical stimulation, but rather by generating an extensor reflex. The most reliable way to elicit this reflex was found to be by inserting needle electrodes into the perineum and stimulating at 30 Hz. This elicited a powerful and reliable extensor reflex which could then be used at the test contraction during HFS experiments. At the termination of the experiment the animal was euthanized with an overdose of pentobarbital sodium.

6.2.2 Chronic pudendal nerve implant and spinalization procedure

The cat was pre-operatively medicated with acepromazine (0.1 - 0.25 mg/kg sc), glycopyrrolate (0.01 mg/kg sc) and buprenorphine (0.01 mg/kg sc) or hydromorphone (0.1 mg/kg sc) and then anesthetized with a mixture of isoflurane (2-3% in carbogen, flow rate 2 L/min). Anesthesia was delivered via a pediatric endotracheal tube. The cephalic vein was catheterized to allow administration of fluids and drugs and a saline drip was delivered throughout the procedure. Body temperature was maintained using a warm water heating pad and the heart rate and SpO_2 were monitored throughout. The implant surgery was performed in a fully equipped, sterile operating room.

The pudendal nerves were exposed bilaterally by incisions lateral to the base of the tail and by blunt dissection through the tissue of the ischio-rectal fossa. Custom-made implants consisting of three stimulus router systems (SRSs) for both the left and right hand sides were implanted (see Figure 6.1 for a schematic of the SRS and a description of the implant). The nerve cuffs were constructed using Silastic tubing (1.02 mm ID, 2.16 mm OD) and Cooner AS636 stainless steel wire. The Silastic tubes were sliced longitudinally and a section of deinsulated wire was inserted through one edge of the tube and exited at the opposite edge. At both the entry and exit from the tubing, a bead of silicone was applied to both insulate the wire and secure it to the tubing. Three such electrodes were placed in each cuff as shown in Figure 6.1. The SRS pick-up terminals were made from stainless steel disks 1.5 cm in diameter and were embedded in a custom made silicone base. The disks were separated by 3 cm and a 1 cm diameter window was cut in the silicone base to expose the metal disk. The array of pick-up electrodes was positioned in the subcutaneous space near the lumbar vertebrae. The electrodes in the nerve cuffs were connected to SRS pick-up electrodes via Cooner AS636 wire. The nerve cuffs were initially positioned on the pudendal nerves bilaterally to include both the caudal rectal and deep perineal branches. Prior to the experiments described here, the implant on the right-hand side was removed and the pudendal nerve was transected. The right-hand side pudendal nerve was damaged by the nerve cuff leading to progressively high stimulation thresholds. Removal of the cuff and transection of the nerve allow stimulation of the left-hand side pudendal nerve without concern for contralaterally induced reflexes.

The SRS used in this implant is a means to transmit electrical stimulation pulses applied to the skin transcutaneously to a subcutaneous system utilizing the resistive and capacitive properties of skin (Gan et al., 2007). The SRS consists of several components: an external stimulator, surface electrodes, and implanted insulated lead wires with conductive "pick-up" and "delivery" terminals. Adhesive gel electrodes (Kendall Soft-E H69P, Mansfield, MA) placed on the skin were positioned directly over the subcutaneously placed pick-up terminals which were connected via insulated lead wires to the delivery terminals inside nerve cuff electrodes. When current pulses were passed be-

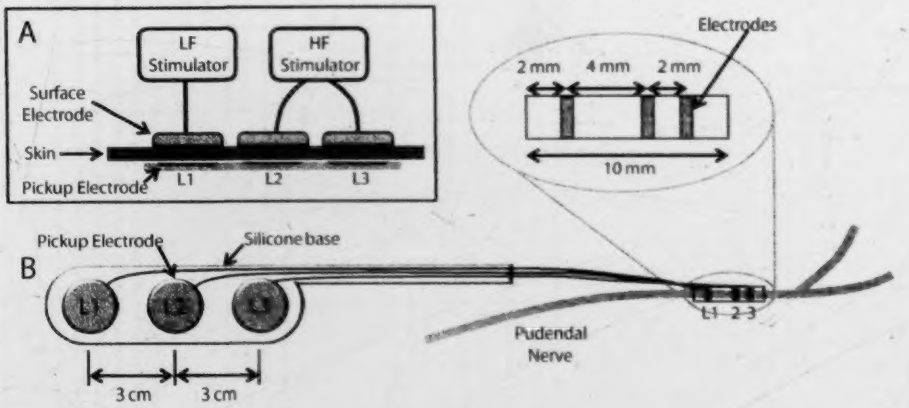

Figure 6.1: *Schematic of the chronic implant*

(A) Diagram of the relationship between surface electrodes and pickup electrodes during a SRS experiment. The proximal surface electrode was connected to a low-frequency stimulator, while the distal electrodes were connected to a high-frequency stimulator. (B) Schematic of the left-hand side chronic implant.

tween a pair of surface electrodes, a portion of the current was captured by the pick-up terminal and routed to the nerve by the insulated lead. In this system, the ratio of internal current (current flowing to the nerve) to the external current (total current delivered by the stimulator) has been shown to be in the range of 0.10 - 0.18 (Gan et al., 2007). Note that the stimulus amplitudes reported here are the external current delivered by the stimulator and not the internal current picked up by the implanted lead and delivered via the nerve cuff.

At the end of the chronic implant, the cat was given ketoprofen (1 mg/kg sc) and hydromorphone (0.07 mg/kg sc) sufficient to maintain a somnolent state. During post-operative recovery the cat was kept warm in a heated cage. Analgesia was maintained by giving two or three additional doses of ketoprofen and/or hydromorphone at 8-hour intervals. Ampicillin was administered for 4 days after surgery, followed by amoxicillin (62.5 mg tablets, 2/day) for 6 additional days.

Twenty-eight weeks after the initial implant, a complete spinal transection at T10 was performed. Pre-operative preparation was the same as described above. The skin was incised over the T10-L1 spinous processes and a laminectomy was performed at the T10/T11 vertebral junction. A transverse incision in the dorsal aspect of the dura mater was made and a solution of 2% lidocaine (0.2 mL) was dripped on the surface of the cord. After two minutes, lidocaine was injected into the spinal cord at progressively more ventral levels. Fine scissors were used to transect the cord. The transection was carefully verified visually with a surgical microscope and a hemostatic mesh, Surgicel (Ethicon Inc., Somerville, NJ), was placed in the gap created by the sectioned spinal cord. The dura mater was closed with 8-0 silk suture and the incision was closed in layers. Post-operative care was the same as described above. After spinal transection, bladder and bowel function were continuously monitored and the bladder was emptied at least twice daily.

Prior to the stimulation sessions, the skin over each pick-up terminal was shaved and cleaned and adhesive gel surface electrodes were applied. An additional adhesive gel electrode was positioned over the base of the tail to act as the indifferent (anode) electrode. Low-frequency pulse trains were delivered between the most rostral surface electrode (L1 in Figure 6.1) and the anode, some of this current being picked up by the underlying L1 pick-up terminal and delivered to the pudendal nerve via the proximal contact in the nerve cuff. Current-controlled charge-balanced HF sinusoids were applied between the L2 and L3 surface electrodes, some of the current being delivered to the nerve via the L2 and L3 pick-up terminals and corresponding delivery terminals in the nerve cuff.

A typical HFS trial consisted of 5 seconds of HFS alone followed by 10 seconds where no stimulation was applied. The initial 5 second segment of HFS was used to evaluate whether HFS by itself had an effect on intraurethral pressure. After this 10 second break, 25 seconds of LF stimulation (LFS) at approximately 30 Hz was delivered through the proximal electrode with 5 seconds of HFS beginning 10 seconds into the LFS train. The response to HFS was evaluated under three conditions: anesthetized with isoflurane, without any anesthesia, and at progressively lower percentages of isoflurane. In the trails where the anesthetic level was varied, the isoflurane vaporizer was initially set to 2.5% and a baseline stimulation set was performed. The anesthetic level was reduced to 2.0% and after 5 minutes the stimulation protocol was repeated. After another 5 minutes had passed, the stimulation protocol was repeated after which the anesthetic level was reduced by a further 0.5%. This procedure was repeated until stimulation could not be continued because the animal had begun to move.

The effect of HFS on external urethral sphincter contractions elicited by proximal stimulation of the pudendal nerve was assessed by measuring intraurethral pressures using a closed-end tomcat catheter (Kendall, 3.5 Fr., Mansfield, MA). The catheter was connected to a Neurolog NL108D4/10 dome and NL108T4 isolated pressure transducer. The urethral catheter was modified by sealing the distal of the two side ports at the tip of the catheter with silicone elastomer. The urethral catheter was also connected to an infusion pump (Pump 22, Harvard Apparatus, Saint Laurent, Quebec, Canada) and during intraurethral pressure measurements, sterile saline was infused at a rate of 0.2 mL/min. The side port of the urethral catheter was positioned in the region of the EUS (typically 4-6 cm from the tip of the urethral meatus). The urethral pressure trace was displayed on an oscilloscope and sampled at a rate of 100 samples per second using a CED 1401 Laboratory Interface and Signal v3 software.

In both the decerebrate and chronic spinal preparations, the response to HFS was investigated with and without anesthesia at stimulation frequencies of 3 kHz and 16 kHz. 3 kHz is within a range where there have been discrepancies in both the modeling and experimental work as to whether or not action potentials are blocked. 16 kHz is in the range where most studies predict that action potential blockade should occur. HFS amplitudes were set to achieve the maximum possible blockade. HFS waveforms were all constant-current sinusoids and amplitudes are reported as half peak-to-peak.

Blockade percent was calculated as follows:

$$Block\ \% = \left(\frac{P_{PreBlockMax} - P_{Block}}{P_{PreBlockMax} - P_{Baseline}}\right) \times 100$$

where P refers to intraurethral pressure.

6.3 Results

6.3.1 Decerebrate preparation

Several key features of nerve conduction blockade were observed in the decerebrate preparation. Before the decerebration had occurred and while the animal was anesthetized with isoflurane, HFS at 3 kHz led to a near complete blockade (84%) of proximally generated muscle twitches followed by immediate and complete recovery of the twitch amplitude (see Figure 6.2A). After decerebration and the discontinuation of anesthesia, the same HFS parameters led to a forceful contraction (see Figure

6.2B). Modeling studies have indicated that if the stimulation amplitude is too low, activation rather than blockade will occur (Williamson and Andrews, 2005; Zhang et al., 2006b). To test whether the block threshold had simply changed after withdrawal of anesthesia, the HFS amplitude was increased from 1 mA to 3 mA. This had no effect on the response. When HFS at 16 kHz was delivered before decerebration when the animal was anesthetized with isoflurane, HFS led to a nearly complete blockade (95%) of proximally generated muscle twitches followed by complete recovery of the pre-block twitch amplitude within 3 seconds (see Figure 6.3A). After decerebration and the discontinuation of anesthesia, the same HFS parameters elicited a similar blocking response (93% complete) with complete recovery of the pre-block twitch amplitude occurring within 1 second (see Figure 6.3B).

In each of these trials, three stimulation pulses were delivered through a distally placed nerve cuff during HFS. The occurrence times of these pulses are indicated by arrows in Figure 6.2 and Figure 6.3. These twitches were used to test whether or not any reduction in muscle force was due to action potential conduction blockade at the site of HFS or some other mechanism. Figure 6.2A clearly shows that the reduction in muscle force elicited by HFS at 3 kHz in the anesthetized cat was not due to a conduction block at the HFS electrode as stimulation of the tibial nerve distally was ineffective in generating muscle twitches. Conversely, HFS at 16 kHz in both anesthetized and unanesthetized conditions eliminated muscle twitches generated by the proximal electrode while having no effect on the ability of distal stimulation of the tibial nerve to elicit muscle twitches (see Figure 6.3). The result of HFS at 3 kHz in the anesthetized cat is further demonstrated in Figure 6.4 where muscle twitches were generated only by stimulation of the tibial nerve distal to the location of the HFS electrode. In this case, HFS eliminated the twitch response even though the electrode stimulating the nerve was more than 1 cm distal to the HFS nerve cuff.

In addition to these trials, we tested the ability of HFS to block tibial nerve activity resulting from extensor reflexes generated using perineal electrical stimulation (13 V, 30 Hz). Figure 6.5 shows the results of this trial when HFS at 16 kHz and 6 mA was delivered to the tibial nerve. Three periods of HFS were delivered, and in all instances, HFS completely blocked muscle contractions. The effect of blocking reflexively generated muscle contractions appeared very similar to the blocking of muscle contractions generated by proximal stimulation of the nerve. Several twitches of the muscle were

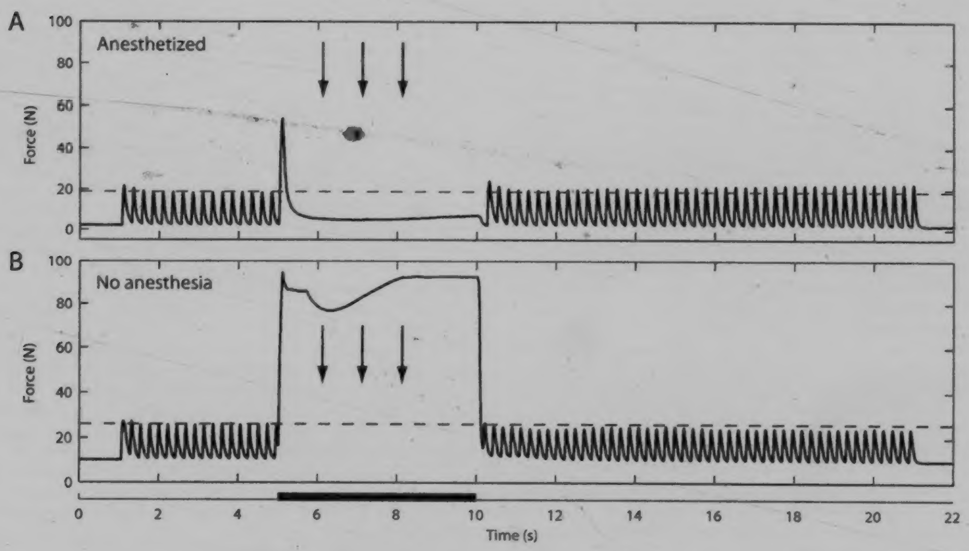

Figure 6.2: *Stimulation of the tibial nerve using 3 kHz, 1 mA HFS*

The stimulation protocols and parameters are identical in A and B. Low-frequency stimulation was delivered through a proximal electrode at 4 Hz beginning at the 1 second time marker. At the 5 second marker, HFS was initiated and lasted for 5 seconds. This duration is indicated by the black bar at the bottom of the figure. During HFS, three stimulation pulses were delivered to the tibial nerve distal to the HFS electrode. The times of occurrence of these pulses are indicated by the arrows. The horizontal gray lines indicate the average maximum twitch amplitude prior to the onset of HFS. (A) Data collected with the animal anesthetized with isoflurane (2.0%). Proximally elicited twitches were abolished by HFS, and distal stimulation of the nerve did not elicit twitches of the triceps surae muscles. (B) Data collected using the same stimulation parameters in the decerebrate cat several hours after the discontinuation of anesthesia. In this case, HFS elicited a large contraction of the triceps surae muscles.

noted after the onset of HFS before a complete block was established. After the cessation of each period of HFS, the reflexively generated muscle contractions returned immediately and at higher amplitudes than before blocking.

6.3.2 Chronic spinalized preparation

In the second experiment, the effect of isoflurane anesthesia on HF nerve conduction blockade was examined in the pudendal nerve of a chronically spinalized cat. A series of stimulation trials were performed in which a standard stimulation protocol was repeated every five minutes at progressively reduced anesthetic levels. 3 kHz and 16 kHz stimulation were again used as the two test blocking

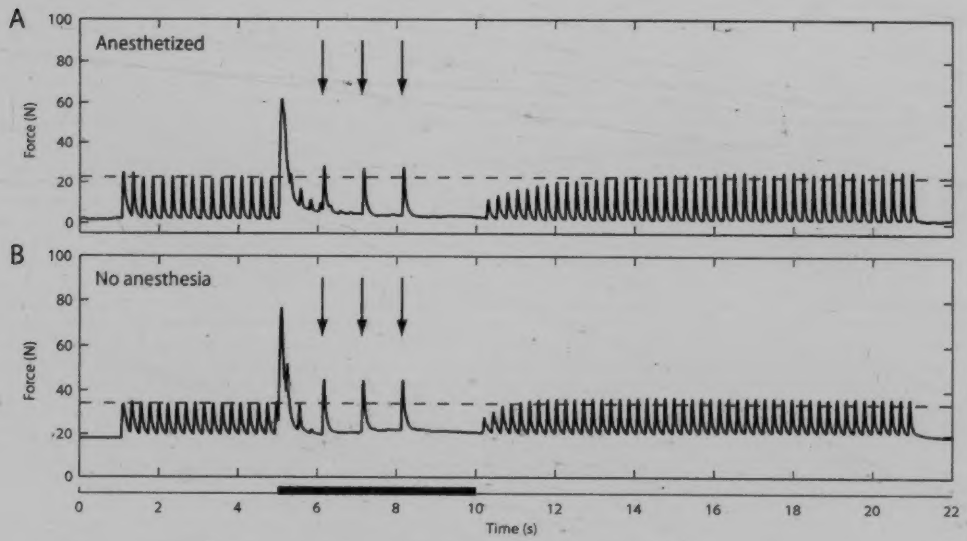

Figure 6.3: *Stimulation of the tibial nerve using 16 kHz, 6 mA HFS*

The stimulation protocols and parameters are identical in A and B. Low-frequency stimulation was delivered through a proximal electrode at 4 Hz beginning at the 1 second time marker. At the 5 second marker, HFS was initiated and lasted for 5 seconds. This duration is indicated by the black bar at the bottom of the figure. During HFS, three stimulation pulses were delivered to the tibial nerve distal to the HFS electrode. The times of occurrence of these pulses are indicated by the arrows. The horizontal gray lines indicate the average maximum twitch amplitude prior to the onset of HFS. (A) Data collected using the same stimulation parameters with the animal anesthetized with isoflurane (2.0%). Proximally elicited twitches were abolished by HFS, but distal stimulation of the nerve resulted in twitches of the triceps surae muscles. (B) Data collected in the decerebrate cat several hours after the discontinuation of anesthesia. HFS continued to abolish proximally elicited muscle twitches while allowing distally generated twitches to occur, unlike the case for 3 KHz stimulation shown in Figure 6.2.

frequencies. A control trial was also performed in which HFS at 3 kHz was used but the level of isoflurane anesthetic was kept constant at 2.5%. This trial was performed to examine whether there was any variation in the effect of HFS on intraurethral pressure over the same time course as those trials where the anesthetic level was varied.

Figure 6.6A shows the block percent for the 3 kHz trials (test and control) and the 16 kHz trial. As the anesthetic level was reduced during 3 kHz stimulation, the block percentage progressively decreased from 91% to 38%. In the 3 kHz control trial, the block percentage decreased from 70% to 46%. However, when at 16 kHz, the block percentage remained fairly constant (95% to 100%) as the

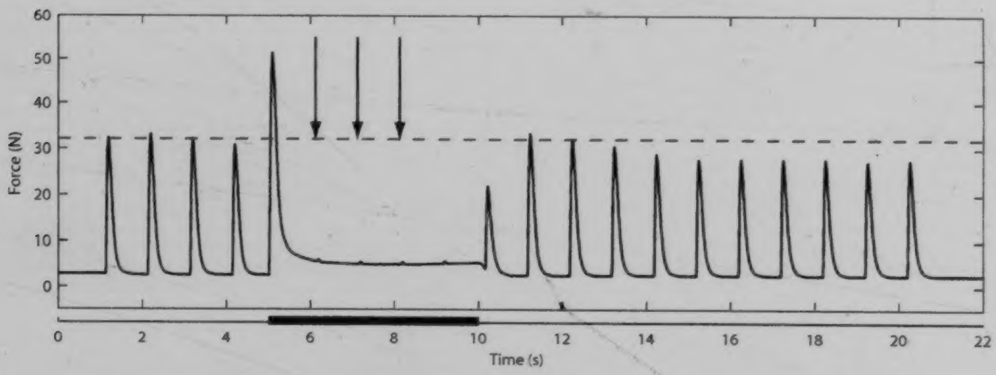

Figure 6.4: *3 kHz stimulation blocks distal twitches under anesthesia*

Muscle twitches elicited by stimulation of the tibial nerve distal to the HFS electrode. During HFS (3 kHz, 1 mA) of the nerve (duration indicated by the black bar at the bottom of the figure), muscle twitches were nearly completely abolished. This record was obtained with the animal anesthetized with isoflurane. The horizontal gray line indicates the average maximum twitch amplitude prior to the onset of HFS.

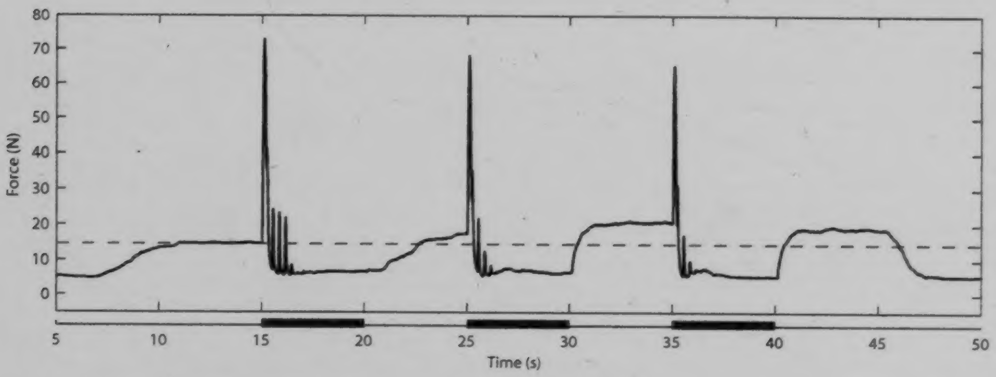

Figure 6.5: *HFS blocks reflexly generated triceps surae contractions*

Contraction of the triceps surae muscles elicited by electrical stimulation of the perineum. Stimulation elicited extensor reflexes leading to steady muscle contractions. Stimulation of the perineum began at the 5 second time marker and ended at the 45 second time marker. The three periods of HFS (16 kHz, 6 mA) are indicated by the black bars at the bottom of the figure. The dashed gray line indicates the amplitude of the pre-block muscle contraction that was reflexively generated by perineal stimulation.

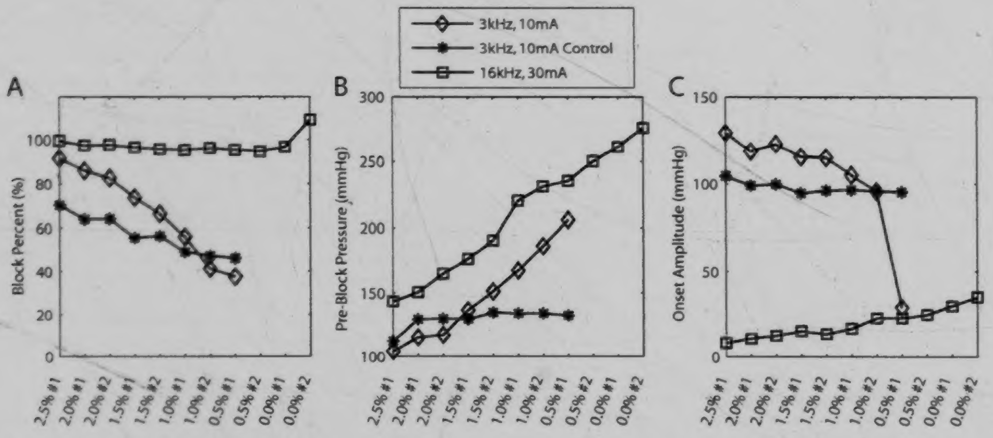

Figure 6.6: *Reducing anesthesia levels affects HFS responses*

The effect of gradually reducing the level of anesthetic on HFS of the pudendal nerve during EUS contractions elicited by proximal stimulation. (A) block percentage, (B) pre-block pressure, and (C) onset amplitude. The anesthetic levels are indicated on the x-axis of each plot for both 3 kHz, 10 mA HFS (open circles) and 16 kHz, 30 mA HFS (open squares). In the 3 kHz, 10 mA control trial (asterisk) the anesthetic level was constant at 2.5% for the duration of the trial. Note that each data point is separated in time by 5 minutes from the previous one.

anesthesia was progressively reduced and actually increased to 110% after the anesthetic had been removed for 10 minutes. The effect of anesthesia on the pre-block pressure is shown in Figure 6.6B. During these trials, increases in intraurethral pressure were elicited by proximal stimulation of the pudendal nerve using low-frequency stimulation at approximately 30 Hz. In the two trials where the anesthetic was reduced, the maximum pressure elicited by low-frequency stimulation increased from 104 mmHg to 206 mmHg in one case (3 kHz) and 143 mmHg to 276 mmHg in the other case (16 kHz). In the control trial, after an increase of 18 mmHg between the first two sessions, there was no change in the intraurethral pressure elicited by low-frequency stimulation for the duration of the trial. The onset response appears to be an inevitable result of HFS and Figure 6.6C shows that when the animal was anesthetized, HFS at 16 kHz consistently elicited a smaller onset response than HFS at 3 kHz. Interestingly, the onset response at 16 kHz increased from 8 mmHg to 36 mmHg as the anesthetic level was reduced while the onset response at 3 kHz decreased from 129 mmHg to 29 mmHg as the anesthetic level was reduced.

Figure 6.7 shows the effect of HFS alone at 3 kHz (test and control) and 16 kHz on changes in intraurethral pressure. Figure 6.7A shows that as the anesthetic level was reduced from 2.5% to 0.5%, HFS alone exhibited a progressive change in its effect on intraurethral pressure. When the cat was anesthetized, 3 kHz stimulation had very little effect on intraurethral pressure. However, once the anesthetic level was reduced to 0.5%, 3 kHz stimulation elicited contractions of the external sphincter leading to increase in intraurethral pressure. During the control trial where the anesthetic level remained constant, 3 kHz stimulation had a similarly small effect on intraurethral pressure for the duration of trial. When 16 kHz stimulation alone was delivered to the pudendal nerve, HFS had no effect on intraurethral pressure at any anesthetic level excepting the onset response, which increased with decreasing anesthetic levels.

6.4 Discussion

In these experiments we have demonstrated that anesthetic can play a significant role in the response of muscle to HFS of peripheral nerve. The primary findings were that HFS at 3 kHz resulted in an apparent block of muscular contraction when animals were anesthetized with isoflurane but resulted in muscle contractions once the influence of anesthetic was removed. In addition, 3 kHz stimulation did not elicit a true axonal conduction block during anesthesia but rather prevented pulses applied distally to the blocking electrodes from causing muscle twitches. Conversely, 16 kHz HFS blocked action potential propagation in both anesthetized and non-anesthetized states, without interfering with the ability of distally applied stimulation pulses to elicit muscle twitches.

Volatile inhalation anesthetics including isoflurane have wide-ranging effects on the nervous system (Krnjevic, 1992). Sodium and potassium channel functions can be directly modified by isoflurane (Duch et al., 1998; Nau, 2008) affecting axonal conduction. Isoflurane also reduces transmitter release at presynaptic terminals (Wu et al., 2004). Some of the recent studies on HFS of the pudendal nerve have used alpha-chloralose as the anesthetic (Bhadra et al., 2006; Boger et al., 2007; Tai et al., 2005c). Much less is known about the cellular mechanisms of alpha-chloralose despite its relativity wide usage in experimental electrophysiology, particularly studies involving autonomic function.

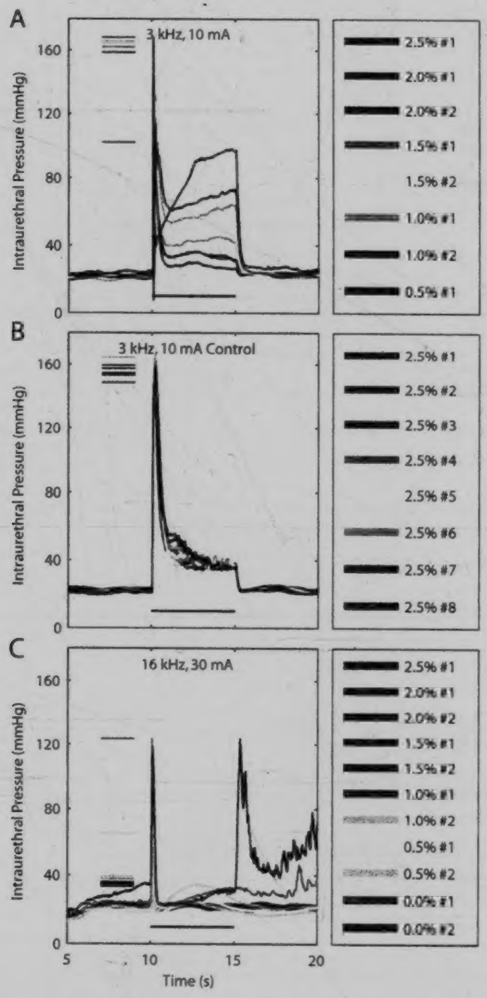

Figure 6.7: *Intraurethral pressure responses to HFS alone at changing anesthetic levels*

The effect of changing anesthetic levels on intraurethral pressure during HFS alone. (A) 3 kHz, 10 mA stimulation of the pudendal nerve had little effect on intraurethral pressure when the animal was anesthetized, but resulted in contractions of the EUS without the influence of anesthesia. (B) 3 kHz, 10 mA stimulation when the anesthetic level was kept constant at 2.5% to act as a time matched control. There was no effect of time on the evoked response. (C) 16 kHz, 30 mA stimulation of the pudendal nerve had no effect on intraurethral pressure at any anesthetic level. In each figure the peak amplitude of the onset response is indicated by a coloured horizontal line associated with each condition. Each trace is separated in time by five minutes from the previous one. Each trial started with the anesthetic level set to 2.5%. The duration of HFS is indicated by the black bar near the bottom of each figure.

The most direct evidence suggests that the anesthetic effect of alpha-chloralose is mediated by an increased chloride conductance as a result of potentiation of the $GABA_A$ receptor (Garrett and Gan, 1998).

Previous investigation of HFS at 3 kHz using experimental techniques or computer simulation has led to conflicting results. Some studies suggested that axonal conduction blockade (Bhadra et al., 2006, 2007; Boger et al., 2007) can occur at this frequency, while in other studies stimulation occurred (Tai et al., 2005c; Williamson and Andrews, 2005; Zhang et al., 2006a). In the present study both blocking and stimulation were observed, depending on whether the animal was anesthetized or not. This occurred in both the decerebrate and chronic spinal experiment, indicating that the phenomenon was independent of the preparations we used. Furthermore, our results revealed that the blocking observed under anesthesia at 3 kHz was not the result of axonal conduction block at the site of delivery of HFS. Since distal stimulation pulses failed to elicit muscle contractions, the reduction in muscle force must have occurred by a mechanism distal to that stimulating electrode. It is unlikely that the reduction in muscle force was due to muscular fatigue as the proximally generated twitches returned as soon as HFS ceased. There are several other sites where neural transmission could have been impaired resulting in a reduction in muscle force. These include axonal branch points, the presynaptic cholinergic vesicles or membranes of the neuromuscular junction and the postsynaptic membrane of the muscle fibres (Sieck and Prakash, 1995).

It is unclear why 3 kHz stimulation should have led to reductions in muscle force in the anesthetized state, but not in the unanesthetized state. Isoflurane has little effect on the function of acetylcholinesterase (Braswell and Kitz, 1977) or muscular nicotinic acetylcholine receptors themselves (Violet et al., 1997). Neurotransmitter depletion also seems unlikely to be the reason for the reduction in muscle force observed under anesthesia as similar depletion should have been observed without anesthesia. In addition, the immediate return of proximally generated muscle twitches after the cessation of HFS makes neurotransmitter depletion unlikely. Isoflurane also reduces vesical release at some central synapses (Wu et al., 2004), and if the same holds true at the neuromuscular junction, it is even less likely that acetylcholine depletion occurred in the anesthetized state. 3 kHz stimulation without the influence of anesthesia led to large contractions of the triceps surae.

These contractions (Figure 6.2B) were likely maximal contractions and were similar in amplitude to the maximum forces measure in triceps surae muscles during jumping in the cat (Walmsley et al., 1978). Kilgore and Bhadra (Kilgore and Bhadra, 2004) demonstrated true action potential block in an isolated frog nerve-muscle preparation using 3 kHz stimulation and verified the block by eliciting muscle contractions with distal simulation. Our results indicate that the isolated frog nerve-muscle preparation may therefore not generalize to the intact mammalian system.

In contrast to our results using 3 kHz stimulation, HFS at 16 kHz elicited a complete block in both experimental preparations with and without the effects of anesthesia. In the chronic spinal preparation, complete blocking of the pudendal nerve was elicited in the awake animal, which is encouraging for the use of this technique in clinical applications. The fact that similar HFS blocking results were observed in two completely different experimental preparations suggest that they may be generally applicable in awake mammals. In experimental protocols, HFS is normally tested for its ability to block nerve activity induced by proximal electrical stimulation of the nerve. Here we demonstrated that HFS at 16 kHz can also block naturally occurring nerve activity elicited by extensor reflexes set up by stimulation of the perineum. This is an important demonstration for potential clinical uses of HFS including treating spasticity. One feature of blocking naturally occurring contractions that was observed was that the magnitude of reflexively generated muscle force during recovery after HFS could be greater than the contraction prior to HFS (see Figure 6.5). It is possible that HFS led to increased spinal excitability resulting in this increased force.

In addition to the main findings discussed above, several other observations of the effects of anesthesia at the two different frequencies are worth mentioning. Firstly, inspection of HFS at 3 kHz under anesthesia (see Figure 6.2A, Figure 6.4, and Figure 6.7A and B) shows that during periods of HFS, muscle contractions did not return completely to baseline values. In fact, HFS alone at 3 kHz elicited a small increase in intraurethral pressure in the chronic spinal experiment. Without the influence of anesthesia, HFS at 3 kHz elicited large contractions. This suggests that small muscle contractions during HFS under anesthesia may be a predictor of very large contractions once anesthesia is removed. Secondly, in the chronic spinal experiment, a progressive change in the amplitude of the onset response was observed as the level of anesthesia was reduced (see Figure 6.6 and Figure 6.7).

Interestingly, as the anesthetic level was reduced, HFS at 3 kHz elicited progressively smaller onset responses while HFS at 16 kHz elicited progressively larger onset responses. Bhadra and Kilgore (2005) reported that increasing the frequency of HFS led to smaller onset responses. This relationship may not hold in the absence of anesthesia.

Given the known mechanisms of isoflurane and alpha-chloralose anesthetics, one might predict that alpha-chloralose would affect axonal conduction and neuromuscular junction mechanisms less than isoflurane. However, the mechanisms of general anesthetics are often multifactorial and challenging to identify. It is telling that the mechanisms of action of isoflurane and other widely used volatile anesthetics are still being elucidated over 160 years after their first introduction into routine medical practice. In addition, if alpha-chloralose had no effect on the mechanism of HFS using 3 kHz stimulation, excitation of the nerve and muscle would be predicted as was observed in awake animals in this study. However, at least in some cases, blocking has been observed (Bhadra et al., 2006; Boger et al., 2007).

It is possible that similar experiments to the ones described here using different anesthetics may provide additional information to help understand the effects of HFS on both axonal conduction and neuromuscular junction activity. From a clinical perspective, it is encouraging to note that HFS at 16 kHz successfully blocked action potential propagation in the absence of anesthesia. To continue the development of HFS nerve blockade as a clinical technique, further experimental studies should address the potential effects of anesthetics on the observations of HF blocking and efforts should be made to test results obtained under anesthetic in chronically implanted conscious animals.

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Chapter 7

General Discussion and Conclusions

"If we knew what it was we were doing, it would not be called research, would it?"

– Albert Einstein

7.1 Discussion and summary

From locomotion to bladder control, the behaviour of our neuromuscular system is remarkably elegant and effortless. This is particularly exemplified once the ability to control the system is impaired due to injury or disease. Despite decades of research, our best efforts to create electrical stimulation systems to exert control over the damaged nervous system still appear simplistic and often crude when compared to the gracefulness of the control imparted by the intact nervous system. In spite of these shortcomings, electrical stimulation techniques have shown great benefits towards improving the lives of individuals. These benefits should only improve as expanding research and commercialization programs in this field will accelerate the development of improved systems in coming years.

The studies presented in this dissertation address the development of electrical stimulation techniques to improve bladder dysfunction, primarily as a result of SCI. Two principle techniques were examined to address this issue: stimulation of the sacral spinal cord with ISMS and high-frequency stimulation of the pudendal nerve. In each case, an original study examining these techniques was followed by a study to further address physiological details of the technique.

7.1.1 History of devices for bladder control

Chapter 2 of this dissertation provides an overview of the history of device usage to manage bladder dysfunction after SCI. Electrical stimulation has been used at nearly every possible location in the nervous system in an attempt to improve bladder function: from penetrating electrodes in the spinal cord to stimulation of the tibial nerve at the ankle. Amongst these techniques the sacral anterior root stimulator (Brindley, 1977) has stood apart as the only implanted electrical stimulation device for motor function to reach regular clinical usage. However, despite its relative success, the device is not widely available and therefore few people who could benefit from the device actually receive one. The success and failures of these devices and techniques provide a reference point from which to consider current research programs and their likelihood of clinical implementation. This point is particularly important to consider from a device development perspective as there is no guarantee that effective devices will become commercially successful devices (Hall, 2003). The sacral anterior root stimulator can be considered a case in point. The device and associated surgical procedures virtually eliminate the need for catheterization, dramatically reduce the incidence of urinary tract infections and restore continence. Yet just over 2,500 people have received these implants despite its being available since the 1970's. In addition to the limited availability of the device, one of the main obstacles is that in order for the sacral anterior root stimulator to operate to maximum effect, recipients must undergo dorsal rhizotomy. Clearly, more than simple efficacy is required for a device to be a clinical success.

7.1.2 Bladder control with ISMS

A major focus of the work in this dissertation was to develop and assess electrical stimulation techniques that have the potential to become clinically available systems in the near future. The approach to the studies was therefore not constrained to a particular electrical stimulation technique or stimulation of a particular region of the nervous system. Rather, the objective was to examine those techniques that seemed most promising. Chapter 3 reports on our experience using ISMS as a technique to recruit specific populations of neurons within the spinal cord to restore micturition. Drs. Blaine Nashold and Harold Friedman were the first to test this technique: initially in animals (Friedman et al., 1972) followed by implants in humans (Nashold et al., 1972). This work was eventually abandoned for a variety of technical and logistic reasons. A large team of clinicians was required for pre-surgical patient screening, surgery was complicated and numerous patients had problems with concomitant sphincter contractions that required additional procedures. In addition, clean intermittent catheterization was becoming established as a safe, effective and simple technique to drain the bladder (Lapides et al., 1972). Finally, initial implants of sacral anterior root stimulators were occurring at a similar time and showed promise (Brindley, 1977). Since this pioneering work however, interest has remained in the concept of stimulating the spinal cord. Independent activation of bladder contractions (Carter et al., 1995; Grill et al., 1999) and ISMS induced urethral relaxations (Blok et al., 1998; Carter et al., 1995) were demonstrated. Combining this knowledge and experience with a newly developed technique of implanting arrays of microwires to deliver ISMS (Mushahwar et al., 2000), it was felt by several research groups as well as the NIH that the time had come to take another look at the clinical viability of ISMS for a bladder control neuroprosthesis.

While the physiologically necessary substrates for a neuroprosthesis were demonstrated in the experiments described in this dissertation, substantial difficulties were encountered both in the technical and physiological aspects of the implants. Details of these difficulties are summarized in Chapter 3. Ultimately, ISMS seems unlikely to form the basis of a neuroprosthesis for bladder control in the near future. However, based on our experience, several general points can be made about the future prospects of ISMS. Intraspinal microstimulation, by its very nature, is a technique fraught with challenges. Pools of motoneurons within the spinal cord are small, are in close physical proximity to motoneuron pools for other muscles (Vanderhorst and Holstege, 1997), and surrounded by cells and *en passant* axons that one does not wish to stimulate. To overcome these difficulties, high-density electrode arrays will be required with perhaps hundreds of electrode contacts to adequately sample the spinal cord and guarantee accurate placement. To make this problem more challenging, the spinal cord is a very soft and mobile structure, making chronic electrode implantation substantially more difficult even than in the cerebral cortex. While there are a number of potentially attractive reasons to pursue ISMS (Guevremont and Mushahwar, 2008), substantial effort will be required to develop the technology to a point where additional pre-clinical evaluation of this technique can occur.

7.1.3 Targets and mechanisms of ISMS

One project that arose from our work with ISMS for bladder control as well as work using ISMS to restore locomotion was focused on understanding the mechanism of ISMS; specifically, what intraspinal neural elements are recruited by ISMS to give rise to observed ISMS responses. A better understanding of the mechanisms of ISMS might guide future work in this area, explain some locomotor related ISMS observations and suggest reasons why ISMS for bladder control might be different than ISMS in the lumbar cord. Chapter 4 described a series of terminal experiments in decerebrate cats that confirmed earlier work that axons generally have lower activation thresholds than motoneuron cell bodies (Gustafsson and Jankowska, 1976), but extended this result by demonstrating that ISMS recruited afferent axons before motoneuron axons even when electrodes were placed deep in the ventral horn. Antidromic activation of afferents, in response to ISMS, was recorded in dorsal root filaments and was detected as far as 17 mm away from the site of simulation in both the rostral and caudal direction. ISMS initially recruited motoneurons transsynaptically and at higher stimulation amplitudes activated motoneuron axons directly.

EMG activity was frequently recorded from multiple, often antagonistic, muscles in response to ISMS through a single electrode at the stimulation amplitudes required for functional movements. It is important to note however that no special care was taken to position the electrodes to elicit single joint movements as would be done in an implant for locomotion. If this careful placement is not done, or if electrodes shifted their position after implantation, our results indicate that ISMS would recruit mixed populations of motoneurons. This is probably because ISMS, rather than activating a focal region of the spinal cord, elicits antidromic afferent activity that can either directly recruit, or lower the threshold of motoneurons over the entire length of the lumbar spinal cord. This mechanism could help explain some previous locomotor-related ISMS observations where single electrodes recruited multiple muscles (Mushahwar et al., 2000, 2002). It also suggests potential mechanistic differences between ISMS in the lumbar and sacral spinal cord based on the afferent innervation patterns of targeted nuclei. Finally, this demonstration has implications for the theory of movement primitives (Giszter et al., 1993) and mechanisms of deep brain stimulation (Dostrovsky and Lozano, 2002).

7.1.4 Pudendal nerve blocking

Given the difficulties encountered with ISMS for bladder control, we began investigating the use of high-frequency stimulation to block action potential propagation in the pudendal nerve. In these experiments, presented in Chapter 5, the primary goal was not to generate bladder contractions, but rather, to block external urethral sphincter contractions that prevent micturition when the bladder pressure is high. A primary goal of this study was to examine whether high-frequency pulse trains could be delivered to the pudendal nerve using a new stimulus delivery system that transmits electrical stimulation pulses through the skin where they are captured by an implant and delivered to the target nerve. We demonstrated that this stimulus delivery system was effective in capturing high-frequency waveforms and could be used to elicit a complete block of action potential propagation in the pudendal nerve of anesthetized cats. One of these cats received a chronic implant and was evaluated over the course of 6 months. High-frequency stimulation continued to effectively block proximally-induced contractions of the external urethral sphincter. In addition to the blocking observed during the study, further experiments were conducted in the conscious animal after complete spinal transection. Blocking was more difficult to achieve and was accompanied by hindlimb reflex activity in some cases. However, in these experiments bladder contractions were frequently observed in response to low-frequency stimulation of the pudendal nerve. This demonstrated that reflexes described in anesthetized cats (Boggs et al., 2005; Tai et al., 2006) are exhibited in conscious, spinal cord injured cats.

7.1.5 Effects of anesthesia on high-frequency stimulation

As a result of differences observed in the blocking efficacy of high-frequency stimulation in the anesthetized and conscious animal, two final experiments were conducted with the goal of determining the role of isoflurane anesthesia on high-frequency blocking. Chapter 6 presents the results of these experiments and shows a distinct difference between the effects of high-frequency stimulation at 3 kHz and 16 kHz on nerve activity. Two independent lines of evidence demonstrated that high-frequency stimulation at 3 kHz appears to block action potential propagation under anesthesia, but elicits strong muscle contractions in the absence of anesthesia. Under anesthesia, 3 kHz produces a neuromuscular junction block rather than axonal conduction block. Stimulation at 16 kHz on the other hand establishes a localized neural conduction block that is quickly reversible with and without anesthesia. Since the mechanism by which high-frequency stimulation blocks action potential propagation is not known, this evidence may help guide future experimental and modeling studies and demonstrates that results obtained under anesthesia may not remain valid in the conscious animal.

7.2 Future experimental directions and clinical feasibility

The results of the work presented in this dissertation suggest a number of avenues for further investigation. ISMS for bladder control was shown to be an unlikely electrical stimulation technique for clinical use in the near future. The primary barrier to further investigating clinical efficacy in animal experiments is the lack of electrodes suitable for chronic implantation in the spinal cord. Developing such electrodes is not a trivial task and would require multi-disciplinary research groups skilled in micro- or nano-fabrication, biocompatibility, low-power wireless technologies and numerous related developments. Given other promising, and technologically simpler techniques such as pudendal nerve stimulation and modifications to sacral anterior root stimulation, it is unlikely that this effort would be worthwhile. However, if electrode development continues as part of a program to develop ISMS as a technique for restoring locomotion, work on bladder control could be worth looking into again at some future time.

Contrary to the difficulties associated with stimulation of the spinal cord, electrical stimulation of the pudendal nerve provided a reliable way to elicit a range of micturition and continence promoting responses. Numerous studies have documented the effects of stimulating branches of the pudendal nerve with the following effects:

1. Inhibition of hyperreflexive bladder contractions by stimulation of the dorsal penile branch of the pudendal nerve in man (Dalmose et al., 2003; Kirkham et al., 2001) and by stimulation of the pudendal nerve trunk in cats (Boggs et al., 2006; Tai et al., 2007).
2. Bladder contractions elicited reflexively by stimulation of the deep perineal branch of the pudendal nerve in cats (Boggs et al., 2005, 2006; Tai et al., 2006, 2007) and by intraurethral stimulation in man (Gustafson et al., 2004).
3. External urethral sphincter contraction elicited by direct stimulation of the pudendal nerve
4. Blocking external urethral sphincter contractions using high-frequency stimulation of the pudendal nerve in cats (Bhadra et al., 2006; Tai et al., 2004).

These four responses represent the complete range of responses required to control the lower urinary tract: inhibition and excitation of the bladder and excitation and inhibition of the external urethral sphincter. Work completed for this dissertation began to examine the use of pudendal nerve stimulation to both block and elicit sphincter contractions in conscious animals. Up to this point, all of the relevant animal work has been done in anesthetized animals. The primary goal for future work on stimulation of the pudendal nerve should be focused on combining multiple techniques from the above list and implementing and testing them in chronically implanted conscious animals. Only after this is complete can the transition to a pudendal nerve implant in humans be justified.

7.3 Concluding comments

130 years after what may have been the first use of electrical stimulation to treat bladder dysfunction (Saxtorph, 1878), and 60 years after research into electrical stimulation techniques to control

the bladder began in earnest (Gaunt and Prochazka, 2006), a device that fulfills all the requirements necessary to restore normal lower urinary tract function after SCI has yet to be developed. However, in the community interested in this problem, there appears to be a critical mass of knowledge, prior experience and new ideas developing focused around electrical stimulation to control the bladder and sphincter. Among the various methods proposed, stimulation of the pudendal nerve, in isolation, or in combination with sacral anterior root stimulation, has the potential to restore all of the major actions required for a functional lower urinary tract. The efforts in this field could lead to the development of a neuroprosthesis that could be tested in spinal cord injured people within five years. The hope is that the work presented in this dissertation will help guide future efforts in this field and contribute to the body of knowledge required to bring a clinical bladder control neuroprosthesis to fruition sooner rather than later.

7.4 Bibliography

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